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The sorption fate of active pharmaceutical ingredients in soils receiving high wastewater inputs and implications for risk assessments

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by

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The sorption fate of active pharmaceutical ingredients in soils receiving high wastewater inputs and implications for risk assessments - Katherine Edith Lees

Population growth, increasing affluence, and greater access to medicines have led to an increase in active pharmaceutical ingredients (APIs) entering sewerage networks. Wastewater in lower and lower middle-income countries use that use wastewater for irrigation may use untreated or poorly treated wastewater resulting in the potential for greater concentrations of APIs to enter soils in this way. Wastewater re-used for irrigation is currently not included in environmental risk assessments for APIs in soils. The addition of wastewater to soils changes the organic content and can increase the pH of soils, which will have an impact on the fate of any ionisable APIs introduced during the irrigation process. As the input of APIs to soil from wastewater irrigation is not currently included in the risk assessments, this is an area that requires increased attention.

A study was undertaken using a modified sorption-desorption batch equilibrium method (OECD 106) to simulate the addition of synthetic wastewater (SWW) to soils compared to a normal OECD 106 study. The APIs studied were ofloxacin, propranolol, naproxen and nevirapine, and represent a range of API physico-chemical properties. These experiments showed that the changes to soil properties (pH and dissolved organic carbon (DOC)) caused by irrigation with SWW can change the fate of APIs in soils. The ionisation state of the API at the altered pH was more important for the positively charged propranolol than it was for the negatively charged naproxen and neutral nevirapine. The K_d and Log K_{oc} increased during the sorption experiment in some cases with SWW. This has implications on the current terrestrial risk assessment where the trigger value for a more detailed soil risk assessment in at Log K_{oc} >4. If the experiment is only performed in 10 mM $CaCl_2$ as is currently required this may lead to unknown risks of APIs in wastewater irrigated soils not being taken into account.

Three soil sterilisation or microbial enzyme suppression methods were investigated to identify how successful they were and if there was any impact on the soil physical chemical structure. Gamma irradiation, autoclaving and the addition of 0.2 g L^{-1} sodium azide were studied. None of the methods successfully sterilised the soils and some changes in soils were identified post-treatment. Autoclaving destroyed the soil structure, turning it into a fine powder and significantly increasing DOC. Sodium azide changed the pH of the loam soil but not the sandy loam soil. Literature suggested that gamma irradiation was the most likely to sterilise the soils with the least amount of disturbance to its physico-chemical properties but increases in DOC were identified in the current study. The changes to soils after sterilisation varied depending on the individual soil properties, indicating that soils should be studied on a case-by-case basis.

Irrigation with wastewater provides continuous inputs of chemicals into soils throughout the growing season so it is vital that more work is done to understand the ultimate fate of pollutants in soil as a result. Wastewater has the potential to change the fate of chemicals in soils meaning that current risk assessments may not thoroughly assess all risks involved.

Author's declaration

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award without prior agreement of the Doctoral College Quality Sub-Committee.

Work submitted for this research degree at the University of Plymouth has not formed part of any other degree either at the University of Plymouth or at another establishment.

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Publications

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Lees, K., Fitzsimons, M., Snape, J., Tappin, A. and Comber, S., "Active Pharmaceutical Ingredients in Soil in Areas with High Water Reuse – Sources, Fate and Environmental Risk Assessment". Postgraduate Society Conference, Plymouth, UK. 2nd December 2015.

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List of abbreviations

AEC	Anion exchange capacity
ANOVA	Analysis of variance
API	Active pharmaceutical ingredient
CDOM	Colloidal dissolved organic matter
CEC	Cation exchange capacity
DDD	Defined daily dose
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
EMA/EMEA	European medicines agency
ERA	Environmental risk assessment
F_{oc}	Fraction of organic carbon
GFF	Glass fibre filters
HPLC-HRAM-MS	High pressure liquid chromatography-high resolution accurate mass-mass spectrometry
HPW	High purity water
K_d	Partition coefficient
K_{des}	Desorption partition coefficient
K_F	Freundlich adsorption coefficient
K_L	Langmuir constant
K_{oc}	Organic carbon-normalized adsorption coefficient
L	Loam
LLMIC	Lower and lower middle income countries
LOD	Limit of detection
LOQ	Limit of quantification
NSAID	Non-steroidal anti-inflammatory drug
OC	Organic carbon
OECD	Organisation for Economic Co-operation and Development
PEC	Predicted environmental concentration
PNEC	Predicted no effect concentration
POM	Particulate organic matter
RO	Reverse osmosis
RPM	Revolutions per minute
RSD	Relative standard deviation
S.D.	Standard deviation
SL	Sandy loam
SWW	Synthetic wastewater
TOC	Total organic carbon
WHO	World health organisation
WWTP	Wastewater treatment plant

1 Pharmaceuticals in soils of lower income countries: physico-chemical fate and risks from wastewater irrigation

This literature review is based on the paper published in Environment International available at http://dx.doi.org/10.1016/j.envint.2016.06.018.

1.1 Overview

Population growth, increasing affluence, and greater access to medicines have led to an increase in active pharmaceutical ingredients (APIs) entering sewerage networks (Kookana et al. 2014). In areas with high wastewater reuse, residual quantities of APIs may enter soils via irrigation with treated, partially treated, or untreated wastewater and sludge (Liu et al. 2013; Dalkmann et al. 2014; García-Santiago et al. 2017). Wastewater used for irrigation is currently not included in chemical environmental risk assessments and requires further consideration in areas with high water reuse (EMEA 2006). This study critically assesses the contemporary understanding of the occurrence and fate of APIs in soils of low and lower-middle income countries (LLMIC), to identify gaps in knowledge that addressing would contribute to the development of risk assessments in LLMIC. The physico-chemical properties of soils vary greatly globally, impacting on API fate, bioaccumulation and toxicity (Kah et al. 2007b; FAO/IIASA/ISRIC/ISS-CAS/JRC 2009). The impact of pH, clay and organic matter on the fate of organic ionisable compounds are discussed in detail. This study identified the occurrence, partitioning and degradation coefficients for APIs in soil:porewater systems. API usage data in LLMICS and removal rates (where used) within sewage treatment plants were identified as key areas where data are missing in order to inform robust environmental risk assessment methodologies.

1.2 Introduction

There has been a global increase in the use of active pharmaceutical ingredients (APIs) in recent decades due to population growth, increasing affluence, changes in disease burdens and easier access to medication (Kookana et al. 2014). In the low and

lower-middle income countries (LLMIC)[1] of Asia, Africa and Central and South America, the use of human pharmaceuticals increased by 23-29 % between 2000 and 2011 (WHO 2011). As a consequence, the loadings of residual APIs and other down the drain chemicals (including personal care products) to soils, surface and ground waters of these countries will increase. The major vector of this loading is wastewater (WHO 2006b; a; d; Corcoran et al. 2010). Wastewater is defined as a combination of one or more of blackwater (excreta, urine, faecal sludge), greywater (kitchen and bathing wastewater), commercial and industrial effluent (including hospitals), stormwater and other urban run-off, and agricultural, horticultural and aquacultural effluent. Each may be fully treated, partially treated or untreated (Corcoran et al. 2010; Jiménez et al. 2010). Difficulties in quantifying the magnitude of wastewater loads, in tandem with a paucity of environmental monitoring data of APIs in LLMIC, makes accurate and precise predictions of temporal trends in API loadings uncertain (Jiménez et al. 2010; Kookana et al. 2014).

Many LLMIC are experiencing physical or economic water scarcity (Figure 1.1) with the former particularly important in northern and southern Africa and southern Asia. Economic water scarcity occurs when there is a lack of investment into water to produce safe drinking or irrigation waters. Physical water scarcity is found when available resources are insufficient to meet all demands, e.g. during a drought (International Water Management Institute 2006). To counter-act shortages of good quality water in arid and semi-arid regions and to conserve its use, many LLMIC use the wastewater they generate for irrigation of agricultural and horticultural land (Corcoran et al. 2010). The water stressed areas of southern Asia produce wastewater in excess of 10×10^9 m^3 yr^{-1} (Figure

[1] Low income countries were defined by the World Bank in 2018 as countries which had a gross national income per capita of <$1005 in 2016 and lower middle income countries had a gross national income per capita of $1006-3995 The World Bank. (2018). Country Classification data. from https://datahelpdesk.worldbank.org/knowledgebase/articles/906519..

1.2) with up to 20 % being used for irrigation (FAO 2015). Other countries such as Israel, Jordan, Syria, Iraq and Mexico use more than 40 % of their municipal wastewater for this purpose (Figure 1.4a). Globally, about 20 million ha of agricultural land is irrigated with wastewater (Scott et al. 2004), with the highest proportions of cultivated areas equipped for irrigation found in the Middle East, southern Asia and western South America, as shown in Figure 1.4(b) (FAO 2015). Per capita daily food consumption requires 2 – 5 m^3 of water (Corcoran et al. 2010), making agriculture a significant requirement for water, particularly in the extensively irrigated regions noted above (Figure 1.3). Irrigation is dominated by untreated and untreated-diluted wastewater, notably in China (> 3.6 million ha), India (> 1 million ha) and Mexico (ca. 190,000 ha), while treated water is extensively used in Chile, Mexico and Egypt (238, 000 ha) (Lautze et al. 2014). Across a range of LLMIC, 80 % of cities use mainly untreated and untreated-diluted wastewater for irrigation (Jiménez et al. 2010). In arid areas, cities such as Dakar (Senegal), Accra (Ghana) and Tamale (Ghana) produce 60-100 % of the consumed leafy vegetables within the city using wastewater irrigation, while 60-80 % of the perishable food for local markets in Hanoi (Vietnam) is produced using diluted wastewater (Drechsel et al. 2006; Corcoran et al. 2010). Water shortages are predicted to become more widespread and acute as human populations increase in number and urbanisation and industrialisation expand, food consumption patterns change, and rainfall distribution and volume alter as a result of climate change (Corcoran et al. 2010; Hanjra et al. 2010). Nevertheless, there appears to be the potential to markedly increase the recovery and re-use of wastewater in many LLMIC, particularly for agricultural use close to highly urbanised areas, given the appropriate incentives (WHO 2006b; a; Jiménez et al. 2010; Lautze et al. 2014).

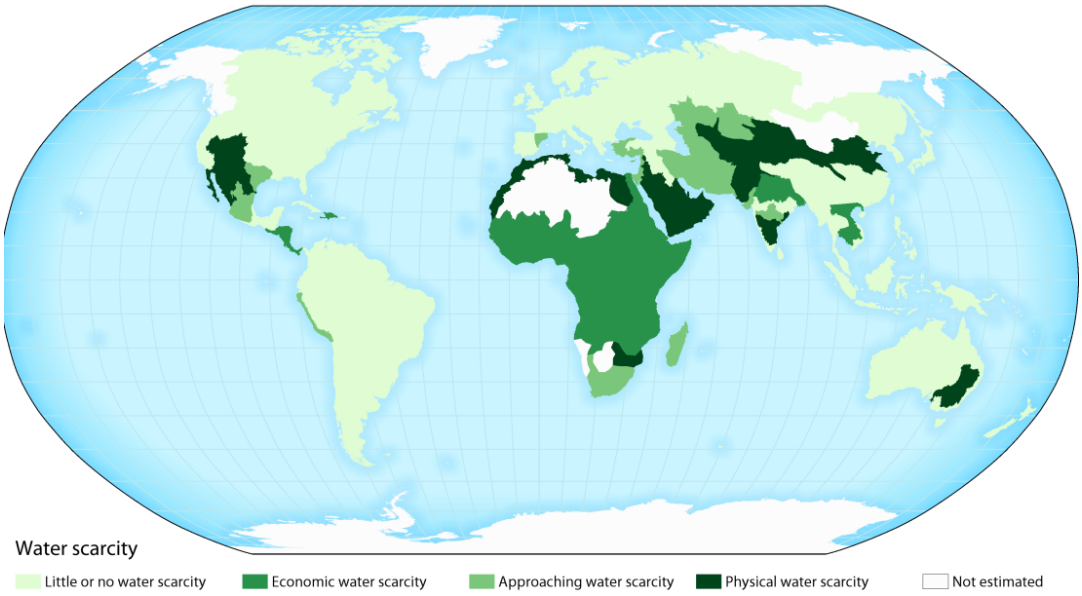


Figure 1.1 - Regions of physical and economic water scarcity. Data from International Water Management Institute (2006).

Definitions:
Little or no water scarcity – abundant water resourced relative to use
Physical water scarcity – more than 75 % of river flows are withdrawn for agriculture, industry and domestic purposes
Approaching physical water scarcity – more than 60 % of river flows are withdrawn
Economic water scarcity – human, institutional and financial capital limit access to water even though it may be available locally to meet demands

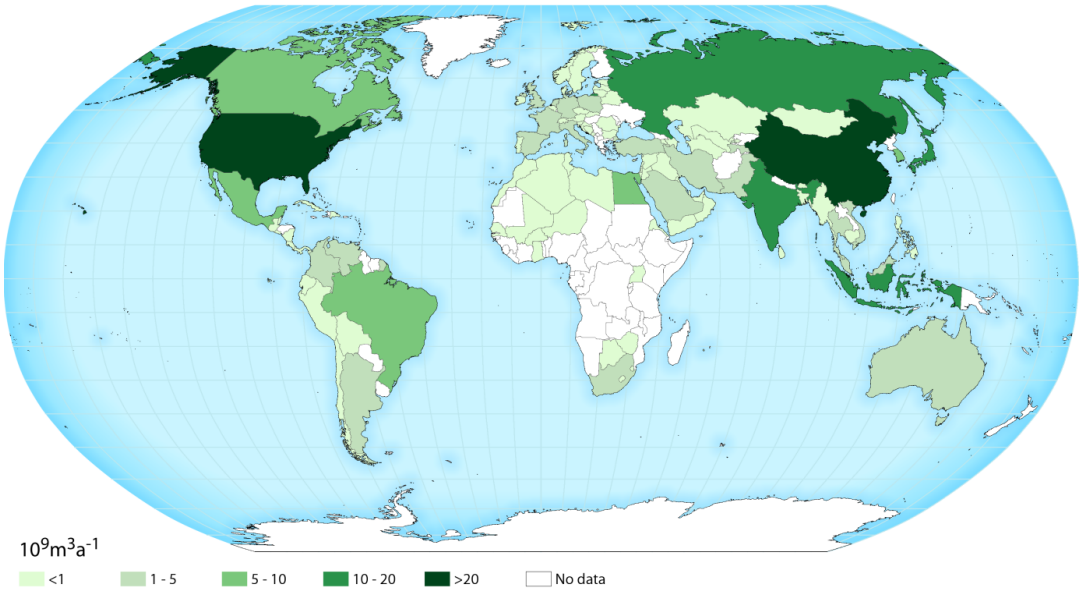


Figure 1.2 - Municipal wastewater production ($10^9 m^3 a^{-1}$) (Data from FAO/IIASA/ISRIC/ISS-CAS/JRC (2009))

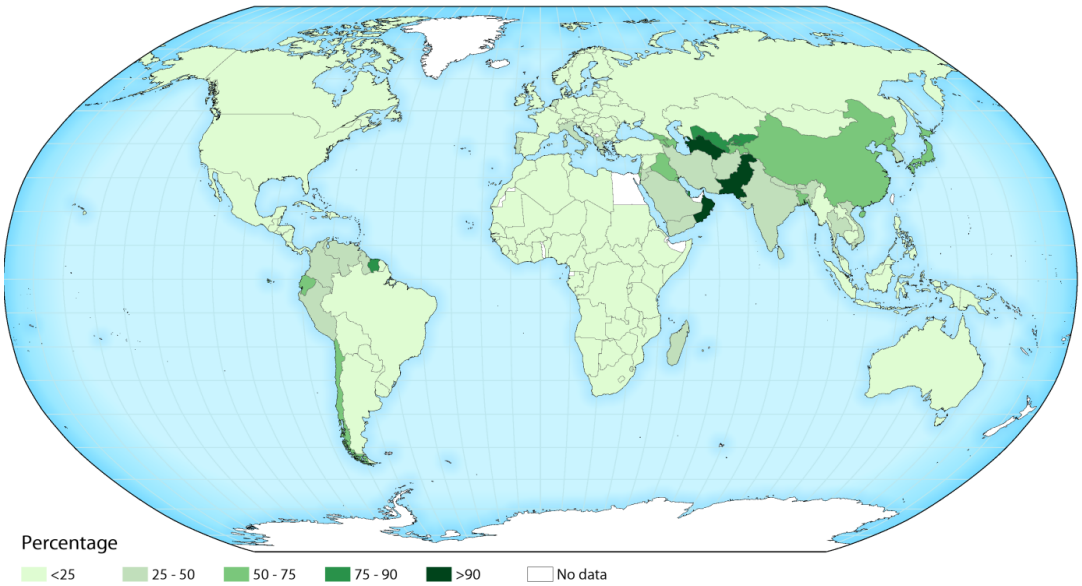
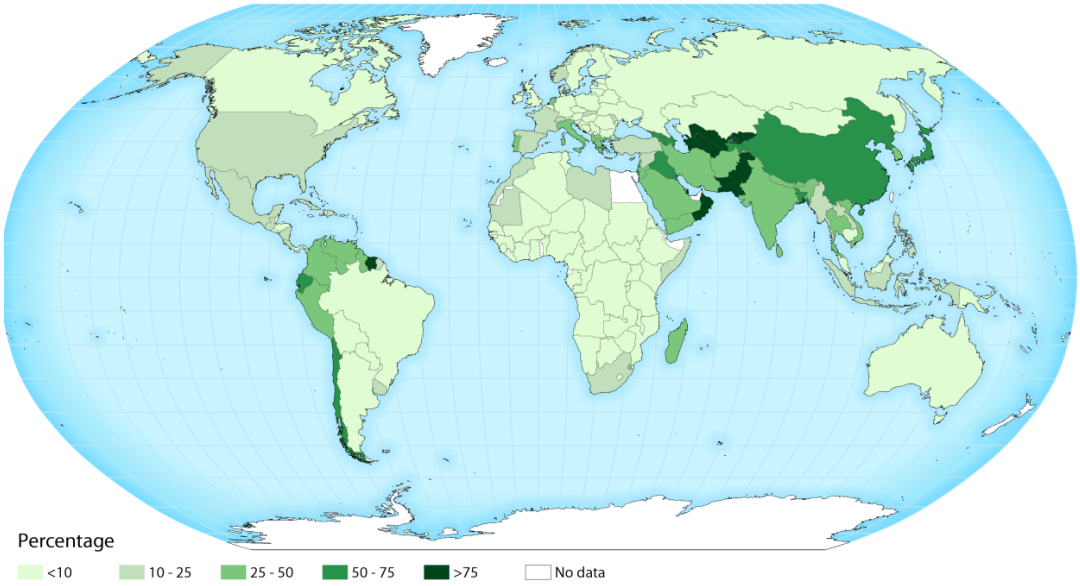


Figure 1.3 - Water withdrawal by agriculture compared to other industries (%) (Data from FAO/IIASA/ISRIC/ISS-CAS/JRC (2009))

(a)



(b)

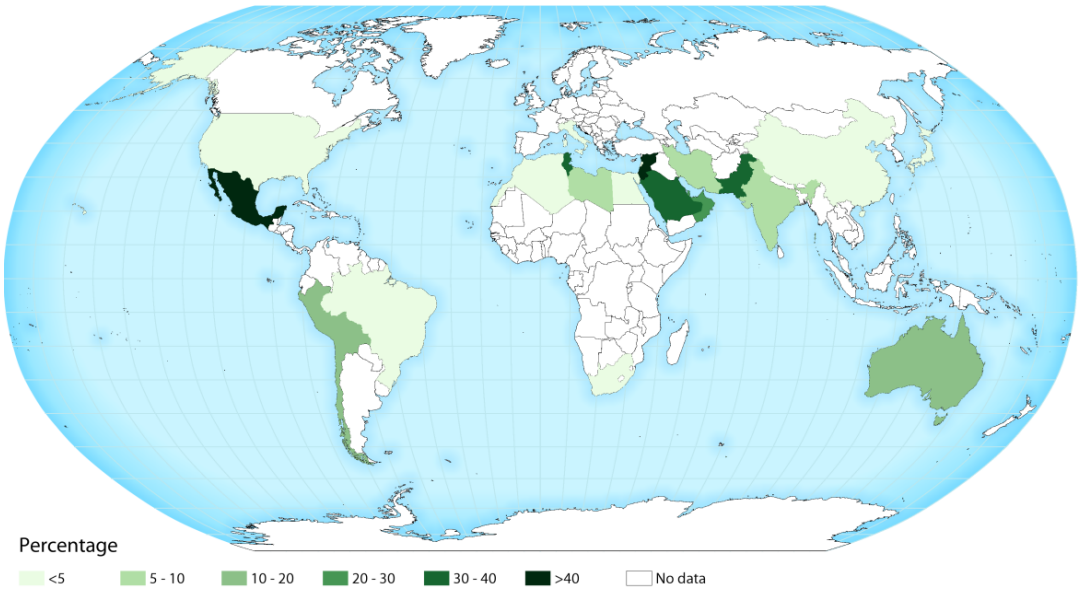


Figure 1.4 - (a) Municipal wastewater used for irrigation (%), (b) Cultivated area equipped for irrigation (%). (Data from FAO (2015))

There is currently a lack of public usage data for the amount and type of APIs used in many LLMIC due to poor record keeping, extensive self-medication and the use of non-prescribed APIs over large population numbers (Kotwani et al. 2012; Kookana et

al. 2014; Rehman et al. 2015). This knowledge gap is further confounded by inconsistent adherence to therapeutic treatments, particularly for longer-term prescribing (Kookana et al. 2014). For some groups of APIs, per capita use may be similar between LLMIC and higher income countries, but owing to larger populations in LLMIC (40 % of the global human population live in China, India, Bangladesh and Pakistan (Rehman et al. 2015)) the actual tonnage used is much greater (Kookana et al. 2014). Usage data are often commercially sensitive and thus unavailable to the wider scientific community; however, projected spending patterns indicate continued expansion of API use in LLMIC (Figure 1.5). In addition, there has been a marked relocation of pharmaceutical manufacturing from high income countries to LLMIC in recent years, with an annual growth of 10 – 15 %, resulting in ca. 13,000 industrial production units in India and China alone (Cardoso et al. 2014a; Rehman et al. 2015). The effluents from these generally poorly regulated sites have been identified as a significant source of APIs to adjacent surface waters and sewage treatment works (Liu et al. 2013; Larsson 2014; Rehman et al. 2015). This can lead to localised 'hot spots' which are manageable if, inter alia, site emissions of APIs are known and safe discharge standards or environmental reference concentrations are developed and enforced (Murray-Smith et al. 2012); nevertheless, there appear to be little data on effluent API loadings (Cardoso et al. 2014a). Thus, it is clear that the paucity of data on API environmental loading from consumption and manufacture is a significant obstacle to the wider understanding of API occurrence, fate and impacts in LLMIC. Concerns regarding persistence and antimicrobial resistance of APIs were highlighted as a priority issue in October 2015 at the International Conference on Chemicals Management led by the United Nations Environment Programme, which called for increased global knowledge of pharmaceuticals in all environmental compartments (Nature 2015). This conference backed the need for global cooperation and awareness to overcome the obstacle of the understanding of API occurrence and fate in LLMIC.

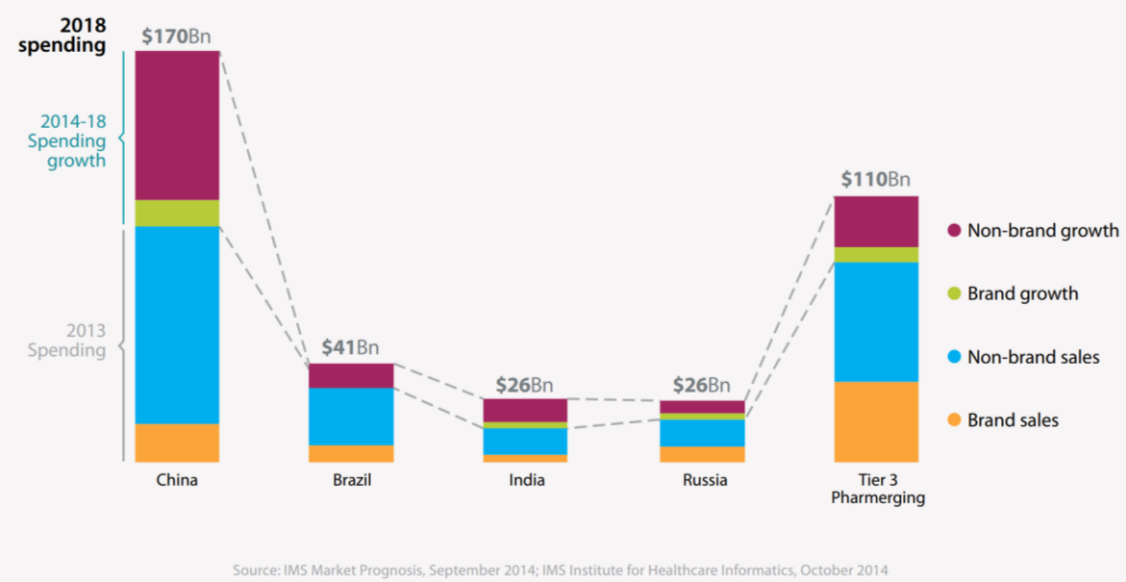


Figure 1.5 - Spending (Billion US dollars) on human medicines in pharmerging countries to 2018.

*Pharmerging countries are expected to see more than $1 billion in absolute spending growth from 2014 to 2018 and which currently have GDP per capita of less than $25,000. Including Algeria, Argentina, Colombia, Egypt, Indonesia, Mexico, Nigeria, Pakistan, Poland, Romania, Saudi Arabia, South Africa, Thailand, Turkey, Ukraine, Venezuela, Vietnam (IMS Institute for Healthcare Informatics 2014).

Guidelines on the safe use of wastewater in LLMIC have been produced since the 1970s, with the most recent published in 2006 (WHO 2006b; a; d; c). The Guidelines are a flexible management framework for safeguarding human health while maximising the benefits of wastewater use in agriculture and aquaculture. The constituents of wastewater addressed with respect to safety in the Guidelines include pathogens, salts, metals, nutrients, acids and bases, suspended matter, organic matter and toxic organic compounds. The last class includes APIs (WHO 2006a; d), but given that the concern regarding these compounds is recent, regulatory frameworks for controlling API loadings (or indeed, other organic contaminants) to soils and waters are rare in LLMIC (Jiménez et al. 2010; Kookana et al. 2014; Sorensen et al. 2015). Indeed, it is only since 2006 that a comprehensive environmental risk assessment has been required for all new marketing authorisation applications for human medicinal products in the European Union (EMEA

2006). The underlying assumption for risk assessments is that wastewater is universally treated in sewage treatment plants (as required under EU law), which, as has been described, does not hold for some LLMIC (Kookana et al. 2014). Furthermore, the European environmental risk assessment framework is only concerned with exposure to API following application of sewage biosolids to soil, and does not include additional scenarios, such as irrigation with wastewater and other contaminated water sources which are relevant to LLMIC and higher income countries. Nevertheless, the environmental risk assessment approach would provide a good framework for LLMIC to adopt, including both Phase I and Phase II (Tier A and B assessments) (Table 1.1). Clearly, the action limits used in Europe (EMEA 2006) for triggering more extensive terrestrial risk assessment by regulators within LLMIC would need to be critically examined to take account of local circumstances. For example, API use and disposal, chemical characteristics and speciation, water re-use, soil physico-chemistry and biology, and climate vary depending on location. It is noteworthy that the appropriateness of some of the action limits in the development of more robust terrestrial risk assessments for APIs in the EU are also under scrutiny, primarily because most APIs (>80 %) can exist either as cations, anions or zwitterions within the pH range covering most surface waters (ECETOC 2013a).

Table 1.1 - Environmental risk assessment pathway for APIs. Trigger values are defined in boxes and once reached ERA moves into the next phase (EMEA 2006)

Phase	Data required to define exposure
Phase I Estimation of exposure	Predicted environmental concentration (PEC) calculated for aquatic compartment
PEC ≥ 0.01 µg L^{-1}	PBT assessment if Log K_{ow} >4.5
	Tailored ERA if mode of action is of concern
Phase II A Initial environmental fate and effects analysis	Ready biodegradability
	Water/ sediment aerobic/anaerobic transformation
Log K_{oc} > 4 in sludge TERA required unless readily biodegradable	Adsorption - desorption using a batch equilibrium method
	Toxicity to algae, *Daphnia sp*. and fish
	Log K_{oc} assessment (> 4 in sludge TERA required)
	Activated sludge respiration inhibition
Phase II B Extended environmental fate and effects analysis (TERA)	Nitrogen transformation test in soil
	Aerobic and anaerobic transformation in soil
	Plant growth
	Earthworm acute toxicity
	Collembola reproduction test

* PBT – persistent bioaccumulating or toxic, TERA – terrestrial environmental risk assessment

The aims of this chapter were to critically assess contemporary understanding of the occurrence of APIs in soils of LLMIC, to identify API sources to soils, to develop a global overview of key abiotic soil characteristics expected to influence the fate of soil-

associated APIs, and highlight the datasets required for the development of a more globally relevant approach to environmental risk assessments that capture exposure scenarios in LLMIC.

1.3 APIs in LLMIC soils: occurrence, sources and factors controlling their fate

The occurrence, sources and fate of APIs in soils following applications of wastewater and biosolids has been an area of concern and study for at least a decade, but the main focus has been on the high income countries of North America and Europe and antibiotic resistance of soil microbes (Thiele-Bruhn 2003; Kinney et al. 2006; BIO Intelligence Service 2013). Similar studies in LLMIC, with the general exception of China, are much rarer (Liu et al. 2013; Kookana et al. 2014; Rehman et al. 2015).

1.3.1 Occurrence in soils

Concentration data for APIs in LLMIC soils are sparse, as a result data shown in Table 1.2 includes other non-LLMIC countries. The majority of APIs identified in studies undertaken were veterinary and human antibiotics (e.g. oxytetracycline, sulfamerazine, norfloxacin), as a result of the combination of high usage in human and animal medicine and concerns about antimicrobial resistance (Gibson et al. 2010; Chen et al. 2011; Li et al. 2011; Rutgersson et al. 2014). Soils in Lahore, Pakistan, were found to have very high concentrations of APIs in soils from fields that were irrigated with wastewater from a pharmaceutical manufacturing plant (Ashfaq et al. 2017). However, generally there is a lack of soil concentration data for LLMICs that host extensive API manufacturing. In arid climates, wastewater for irrigation is added continuously throughout the year, which, in principle, can lead to accumulation of API in the soil and leaching of APIs through soil profiles to groundwater even for readily biodegradable compounds. In soils irrigated with untreated wastewater in the Tula Valley (Mexico), Gibson et al. (2010) calculated that carbamazepine and triclosan had accumulated in upper soils horizons significantly. The

concentration patterns of both compounds were highly and positively correlated with the soil organic matter concentration, suggesting that this soil component was a critical factor in their accumulation. For other APIs studied (ibuprofen, naproxen and diclofenac) there was no evidence of accumulation, probably due to biodegradation rates exceeding application rates.

Table 1.2 - Concentrations (µg kg^{-1} dry weight) of APIs in soils of countries that use wastewater irrigation

Location	API	Concentration (µg kg^{-1})	Reference
Pearl River Delta (China)	Oxytetracycline	9.6 ± 22.9	(Li et al. 2011)
	Sulfamerazine	16.0 ± 20.4	
	Norfloxacin	61.9 ± 33.1	
Hebei (China)	Salicylic acid	4.5 ± 0.8	(Chen et al. 2011)
	Oxytetracycline	6.2 ± 0.2	
	Tetracycline	6.9 ± 0.5	
Pearl River Delta (China)	Tetracyline	5-22	(Pan et al. 2018)
	Sulfamethazine	1-5	
Tula Valley (Mexico)	Ibuprofen	< 0.1 – 0.30	(Gibson et al. 2010)[a]
	Naproxen	< 0.20 – 2.40	
	Carbamazepine	0.1 – 16.4	
	Triclosan	0.4 – 35.5	
	Diclofenac	< 1.0	
Baithole (India)	Ciprofloxacin	0.014	(Rutgersson et al. 2014)
	Norfloxacin	0.011	
	Ofloxacin	0.019	
Lahore (Pakistan)	Ibuprofen	321-610	(Ashfaq et al. 2017)
	Diclofenac	101-257	
	Naproxen	30-199	
	Ofloxacin	15-30	

[a] Concentration range reflects 5 soils and variable sampling depths

1.3.1.1 Wastewater application to soils

In many LLMIC the proportion of the population living in an urban environment is smaller than in high income countries and sewerage connectivity is much lower (Kookana et al. 2014). In addition, sewage treatment plants often underperform or are underused (Kookana et al. 2014). Little of the non-urban population is connected to sewerage systems, instead relying on septic tanks, pit latrines and other low technology systems. As a consequence, in LLMIC, as a whole, ca. 90 % of untreated and poorly treated wastewater is discharged directly to surface water and soils Figure 1.6(UN Water 2008; Kookana et al. 2014; Sorensen et al. 2015). The surface water contaminated with wastewater may be subsequently used for irrigation.

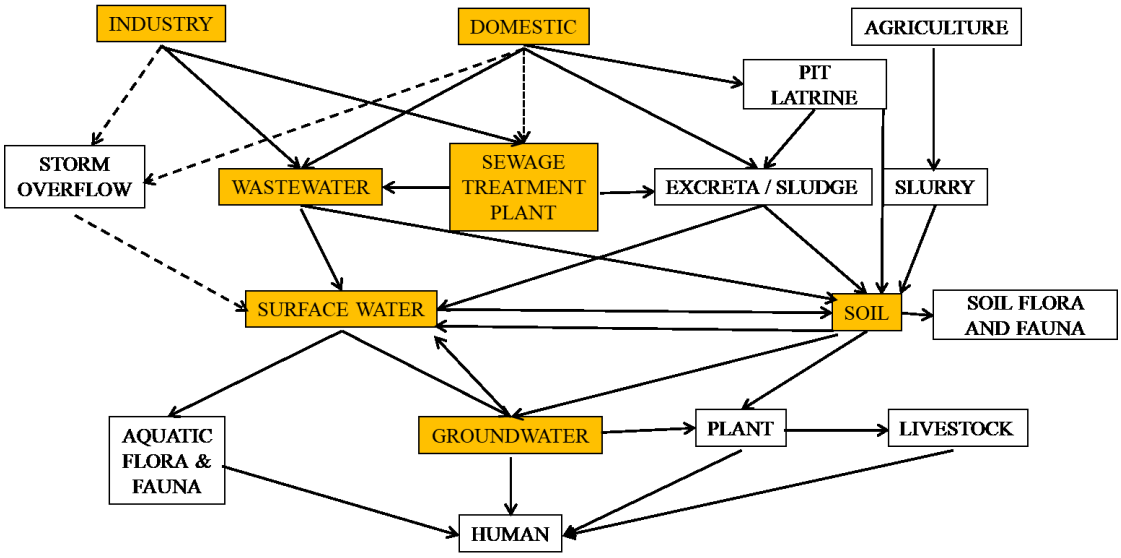


Figure 1.6 - Potential flows of APIs in the environment from their sources in the industry, domestic and agricultural sectors. Dotted lines indicate less important pathways in LLMICs, shaded areas represent flows discussed in the text (UN Water 2008; Kookana et al. 2014; Sorensen et al. 2015).

Removal efficiencies of APIs in sewage treatment plants globally depend on their concentration in the influent, their physico-chemical properties, the method of treatment and the operating conditions (hydraulic/sludge residence times and the weather/climate

during processing). Variation in removal efficiencies can also be the result of sampling factors that need to be assessed before using data for risk assessments or modelling. These include type of sampling (e.g. grab samples or composite sampling schemes) and the inclusion of hydraulic retention times when sampling.

The most commonly reported treatment type is activated sludge as it is one of the most commonly used treatment type used globally (Melvin et al. 2016). In Bangkok (Thailand), sewage treatment plants with different activated sludge treatment processes achieved a wide range of API removal efficiencies, from 19-90 % (grab samples). For example, diclofenac showed variable removal (19-60 %) while atenolol removal was higher (76-90%) and more consistent (Tewari et al. 2013). This variation has also been identified in Europe; in European Union countries, 0-98 % removal of APIs has been measured (Castiglioni et al. 2005; Gardner et al. 2013). Seasonal differences in API concentrations in the receiving waters were found between the high flow in January (1-148 ng L^{-1}) and low flow in September (<1-1100 ng L^{-1}) owing to dilution, indicating that climactic variations must also be taken into account when assessing the efficiency of sewage treatment plants and dilution in the receiving environment (Tewari et al. 2013). Sewage treatment plants in Korea, a high income country, showed a similar variation in API removal efficiencies, with a wide range for carbamazepine removal (42-83%) and a narrow one for naproxen (72-88%) (grab samples) (Sim et al. 2010; The World Bank 2018). Treatment types commonly used in rural areas globally include lagoons and oxidation ditches (Melvin et al. 2016). In rural Australian communities using a series of 10 lagoons for sewage treatment, high removal efficiencies were measured for naproxen (90%) and diclofenac (90%) (composite sampling scheme) (Ying et al. 2009). In this study degradation of the APIs was suggested to be the primary removal mechanism due to long residence times and the removal of sludge and solid sedimentation in the early stage lagoons. Whilst Australia is not a LLMIC, sewage processing in rural communities

is relevant due to the arid climate and simple sewage infrastructure. It can be concluded that further studies into global removal efficiencies for a wider range of APIs and treatment processes are required.

Concentrations of APIs in effluents from the pharmaceutical industry in LLMIC can be a significant source of APIs if discharges are poorly managed, as API levels in the effluent can be orders of magnitude higher than those in urban sewage effluent (Cardoso et al. 2014b; Larsson 2014). In China and Korea, industrial wastewater is often mixed with domestic wastewater prior to discharge to the sewage treatment plant in order to enhance biodegradation of organic contaminants, while in India, Pakistan and Bangladesh, industrial wastewater is more often discharged directly into surface waters (rivers) or domestic sewage systems where these exist (Rehman et al. 2015). Despite the potential for ecotoxicological impacts, there appear to be few data on industrial effluent API loadings in LLMIC (Larsson 2014).

1.3.1.2 Other sources of APIs to soils

While the main soil source of human APIs focused on in this thesis is from wastewater irrigation, it is important to be aware that other soil sources are also important. These include; sludge application to land, runoff from nearby fields that have been treated with sludge or wastewater, direct excretion onto soils, pit latrines and waste disposal (Li 2014; Lu et al. 2016). Two of these sources are discussed in further detail below.

Aside from wastewater, sewage sludge input to agricultural lands in one of the largest sources of APIs to soils (Li 2014; Verlicchi et al. 2015). Sewage sludge is added to soils to improve the quality and structure for growing crops and to reduce the dependence on artificial fertilisers (Kinney et al. 2008). Concentrations of APIs in sludge various between treatment type, country, and source of untreated sewage. In North America maximum concentrations of about 5000 $\mu g\ kg^{-1}$ have been measured (Kinney et

al. 2008). In Slovakia the concentration of 93 APIs were investigated in sewage sludge with 52 of the target compounds being quantifiable, the maximum concentration detected was similar to the North America study at 5600 μg kg^{-1} for fexofenadine (Ivanová et al. 2018). These two examples in economically developed countries indicate that the environmental risk from APIs as the result of applying sludge to agricultural land should not be overlooked and is already the main focus of ERAs of APIs.

Landfill leachate has the potential to pollute soils and groundwater with many different contaminants due to the large mixture of different waste that is disposed of to landfill (Lu et al. 2016). Downstream leachate from landfills in Taiwan have been studied with 15 out of 26 APIs were detected at quantifiable concentrations, the maximum concentration found was 5.9 μg L^{-1} for ketamine, most others were found in the mid 100s of ng L^{-1} range (Lu et al. 2016). A study in North America found APIs in leachate from landfills up to a maximum concentration of 47.9 μg L^{-1} for the non-prescription API lidocaine from a study that included 55 APIs (Masoner et al. 2016).

1.3.1.3 Physico-chemical factors controlling the fate of APIs in soil

Many APIs are designed as ionisable compounds to ensure that active components of the administered dose reaches the specific target location within the human body (Küster et al. 2014). The fate and toxicity of ionisable organic contaminants in soils is therefore significantly influenced by soil pH, soil temperature the concentration and type of organic matter and clay (and hence ion exchange capacity), the lipophilicity of the API (described by the n-octanol-water partition coefficient (Kow), and its strength as an acid or base (described by the acid dissociation constant, Ka) (Küster et al. 2014). Nevertheless, other factors, such as soil aeration, moisture content, temperature and patterns of API use, form emitted and mode of emission (episodic or continuous) will also play a role (Jjemba 2006; Dalkmann et al. 2014). More than 80% of APIs in the Australian

Medicines Handbook (>900 APIs) with a molecular weight < 1000 Da that are not mixtures or salts have been estimated to be ionisable at environmental pH (5-9) (Manallack 2009). The distribution of ionisation of acids and bases with a single ionisable group with pK_a of 2-10 at pH 2-10 is shown in Figure 1.7. At low pH values all of the basic APIs are predicted to be ionised and very small proportions of the acidic APIs are predicted to be ionised to some extent, this trend shifts the opposite way at pH 7 (Charifson et al. 2014). Therefore, predicting how changes in the soil environment influence the ionisation of the molecule and its resulting lipophilicity and hence its behaviour and fate (accumulation, abiotic and biotic degradation, leaching), is an area of ongoing research with respect to exposure assessment and environmental risk assessment (Boxall et al. 2012; ECETOC 2013a). The ambient conditions of soils will vary with location and climate, which makes the direct knowledge transfer of API behaviour and fate in soils in high income countries, where most studies have been undertaken, to LLMIC less straightforward than perhaps anticipated. In the following sections, API – soil particle interactions and the potential roles of soil pH, clay and organic matter in relation to API fate in wastewater used for irrigation by LLMIC are examined in more detail.

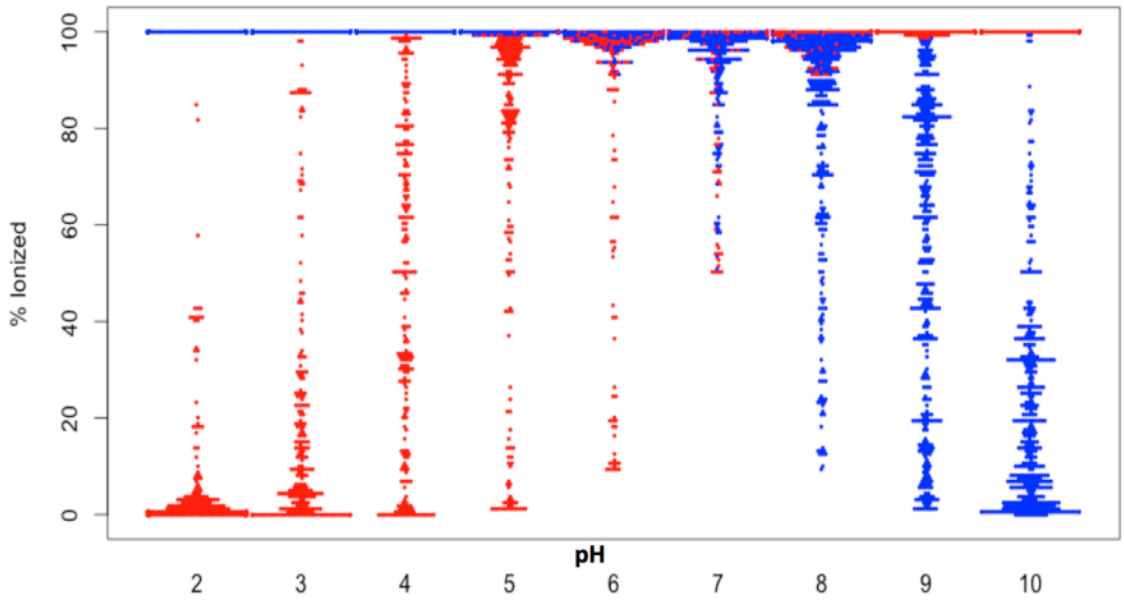


Figure 1.7 – Distribution of percentage ionisation of a range of APIs (661 APIs), which have a single ionisable group at different pH values. Acidic compounds are red and basic are in blue. Figure is adapted from (Charifson et al. 2014).

1.3.1.4 API – soil particle interactions

The range of physico-chemical interactions that may occur between an API and soil particles are summarised in Figure 1.8 for propranolol (Schwarzenbach et al. 1993). This API has a pKa of 9.45 so at pH 7 the cation will be 282 times more abundant than the neutral form of the molecule. The extent of ionisation and the charge on the ionised molecule will affect the extent to which these reactions occur. In reaction 1, the protonated propranolol molecule forms an ionic bond with a negatively charged surface group on the particle. With reaction 2, a free (i.e. unprotonated) molecule reacts with a functional group on the particle to form a covalent bond. For reaction 3, the naphthoxy side chain of propranolol undergoes hydrophobic interactions with particulate organic matter (POM), or the cation can bond with negatively charged functional groups within the POM. For the final reaction, 4, van der Waals forces and dipole–dipole interactions may be involved in sorption. Other processes which may influence interactions of APIs with particles include ligand exchange and hydrogen bonding (ECETOC 2013b).

In contrast to propanolol, naproxen (pKa 4.15) ionises to an anion and at pH 7 the anion will be 708 times more abundant than the neutral molecule; interactions with anionic surface exchange sites would therefore be negligible but it may form a hemi-acetal through carbonyl addition to the reactive surface group. Anioic APIs may also be subject to cation bridging to form interactions with soil surfaces (mechanism 5 on Figure 1.8) (MacKay et al. 2005). This process is where the anionic or polar functional group become bound to a metal cation absorbed by a negatively charged mineral surface, for example, the negative structural charge on clay minerals and magnesium oxides or ionised surface OH (Sposito 2008). One final mechanism that is important for neautral molecules is the movement of hydrophobic neutral molecules into natural organic matter to escape an aqeous solution (Schwarzenbach et al. 1993).

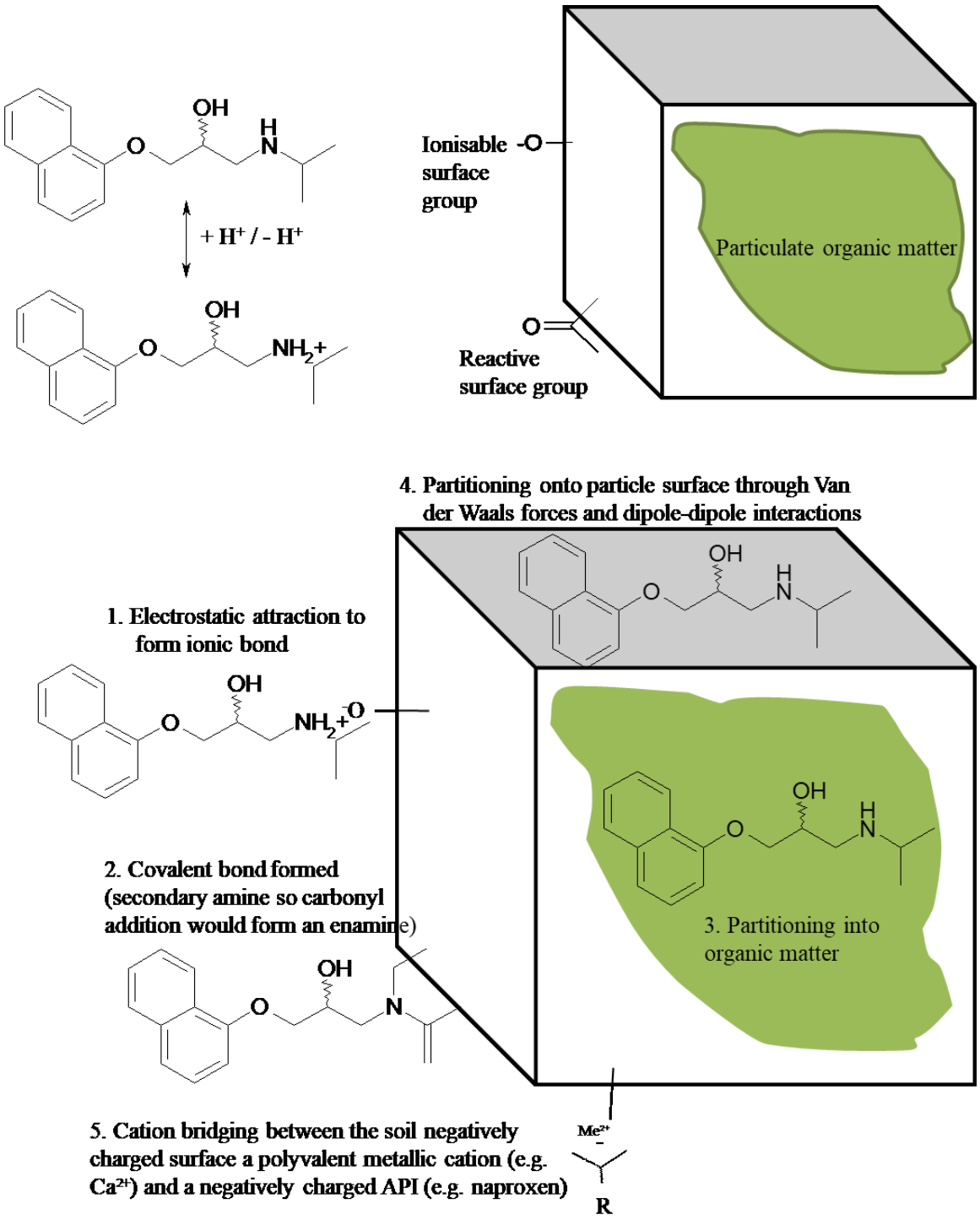


Figure 1.8 - An example of the possible sorptive interactions between the API propranolol (1-4) and a heterogeneous soil particle that may control partitioning of the molecule between the dissolved and particulate phases in soils. Cation bridging has been shown for a anionic API (%) (adapted from Schwarzenbach et al. 1993)

1.3.1.5 Soil pH

The total potential acidity of a soil essentially comprises the activity of protons in soil pore water plus exchangeable protons at the surfaces of the soil particles (Kah et al. 2007b). Figure 1.10 shows the global distributions of soil pH, determined in a water/soil suspension (FAO/IIASA/ISRIC/ISS-CAS/JRC 2009). While the water method for pH measurement will not necessarily account for exchangeable protons (Kah et al. 2007b), top soils (0-30 cm) in LLMIC using wastewater irrigation (Figure 1.4 (a)) generally fall into the pH range 5.5-8.5, with soils in southern China, eastern India, and Bangladesh more frequently in the range 4.5–5.5. Wastewater is usually slightly alkaline, which will mitigate the generally acidic nature of the soil environment to some extent (WHO 2006d).

Soil pH will influence the net charge on ionisable APIs and they will be fully ionised (> 99 %) when the pH is at ± 2 pH units from their log K_a (pK_a) values, as shown in Figure 1.9; thus many APIs will be significantly ionised at typical soil pH. APIs with more than one pK_a value will exhibit additional charge complexity.

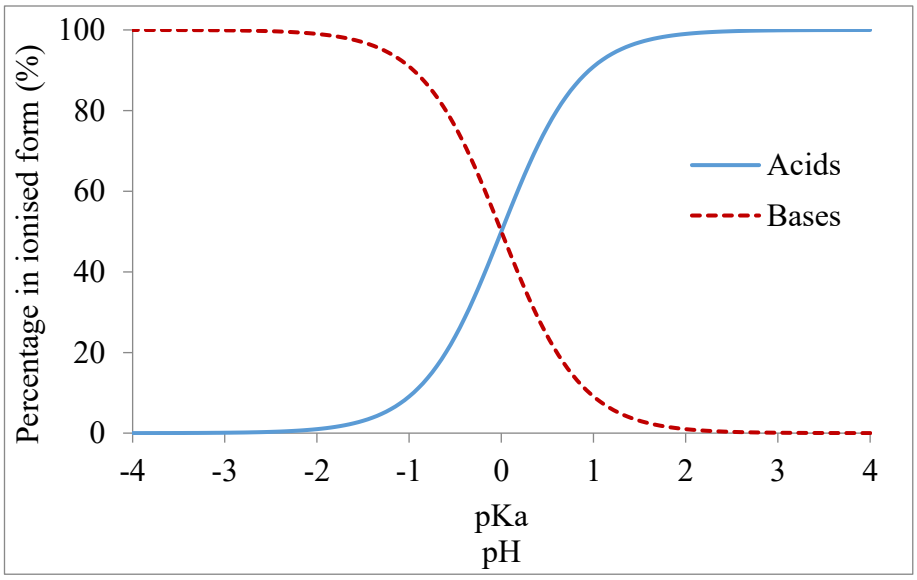


Figure 1.9 - Percentage of acidic or basic API in ionised form as pH varies from less than 4 units to greater than 4 units from the compound pKa (ECETOC 2013a).

Ionic APIs will be less lipophilic than the neutral forms and hence more water soluble; nevertheless, cationic (basic) APIs may be expected to sorb to negatively charged components within the soil, such as clay and organic matter (Franco et al. 2009; Lertpaitoonpan et al. 2009). With reference to Figure 1.8, propranolol (pKa 9.3) will be cationic at most soil pH, and is expected to sorb to the negatively charged components of soils via electrostatic attraction (ter Laak et al. 2006a; Schaffer et al. 2012). In contrast, acidic APIs, such as naproxen (pK_a 4.2), will be in anionic form at soil pH higher than the pK_a; thus a reduction in electrostatic sorption at > pH 5 may be the result of repulsion between the anionic API and the negative charge on the soil (Paul et al. 2013). Fluoroquinolone antibiotics have two pK_a values (e.g. ofloxacin, pK_a 5.97 and 8.28) which at environmental pH tend to be zwitterionic, but can also be cationic, anionic, or uncharged (Vazquez-Roig et al. 2012). At pH $\leq$ 5 ciprofloxacin (pK_a 6.18 and 8.76) electrostatic sorption can be hypothesized to increase as pH decreases due to the cationic form of the API interacting with the negatively charged soil surfaces. At pH $\geq$ 5, ciprofloxacin sorption will decrease due to diminishing cationic charge and an increase in the anionic carboxylic acid moiety within the net neutral zwitterion, leading to repulsion from the negatively charged soil components (Vasudevan et al. 2009).

The more acid soils found in parts of Asia, noted above, are relatively rich in positively charged Al and Fe sesquioxides (FAO 2014) which may provide sorption sites for anionic (acidic) APIs (Hyun et al. 2004; White 2013). Soil pH will also influence the pH-dependent charge on the organic matter, clay minerals and metal sesquioxide components of the soil, which in turn might be expected to influence API sorption (Hyun et al. 2004).

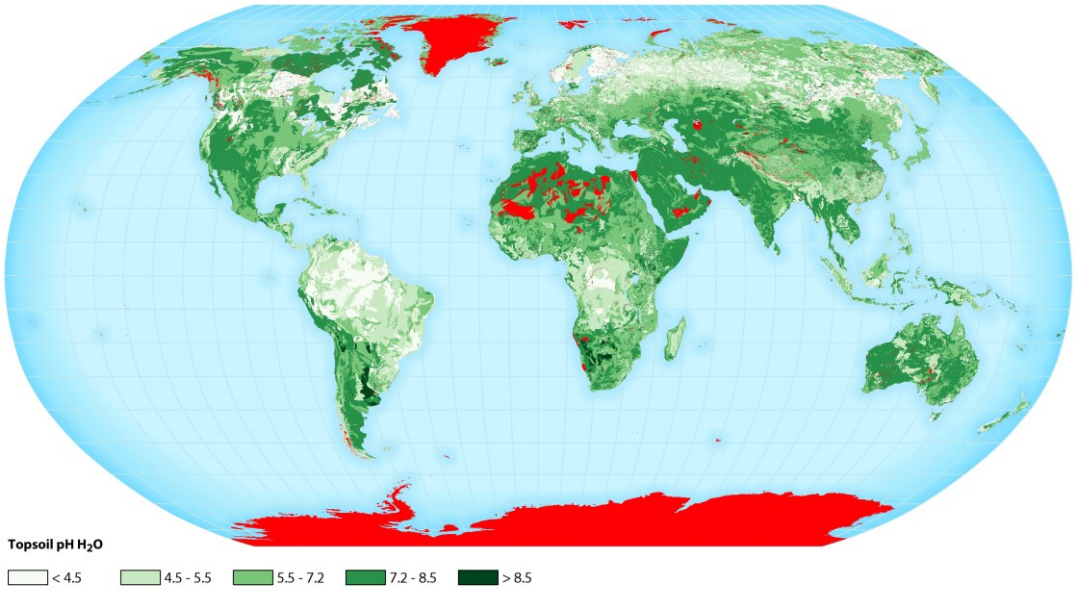
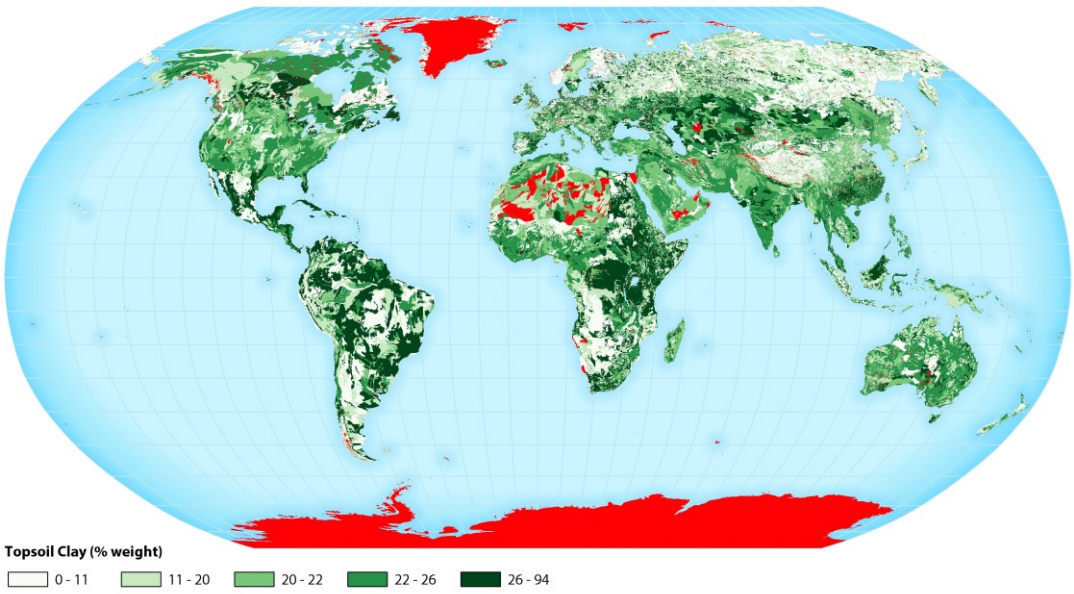


Figure 1.10 - Global distribution of soil pH (0-30 cm depth) (Data from FAO/IIASA/ISRIC/ISS-CAS/JRC (2009)). Coverage in red indicates no data.

1.3.1.6 Soil clays

Clays are essentially aluminosilicate minerals with a particle size of less than 2 µm. Figure 1.11(a) shows the distribution of the clay content in surface soils (defined as 0-30 cm depth) across the globe (FAO/IIASA/ISRIC/ISS-CAS/JRC 2009). The LLMIC that use wastewater for irrigation, as shown in Figure 1.4(a), have soils with a wide range of clay content. In China, the south eastern regions have contents of 30-45 %, falling to mainly 15-30 % in the north, while soils with < 15 % clay are more frequent in the western areas. Soils in the central region of India have high clay contents (45-60 %), with some in the 30-45 % range. In the coastal zones of India, in Bangladesh and in parts of Pakistan, 15-30 % clay is more common. Lower clay contents (< 15 %) are found in northern India and central Pakistan. In north Africa, soils with clay contents 15-30 % are common, falling to < 15 % in the eastern areas. Soils along the western coastal zone of South America have clay contents in the range 15-30 %. Elsewhere in the sub-continent and also in Mexico, soils with a wide range of clay content occur.

(a)



(b)

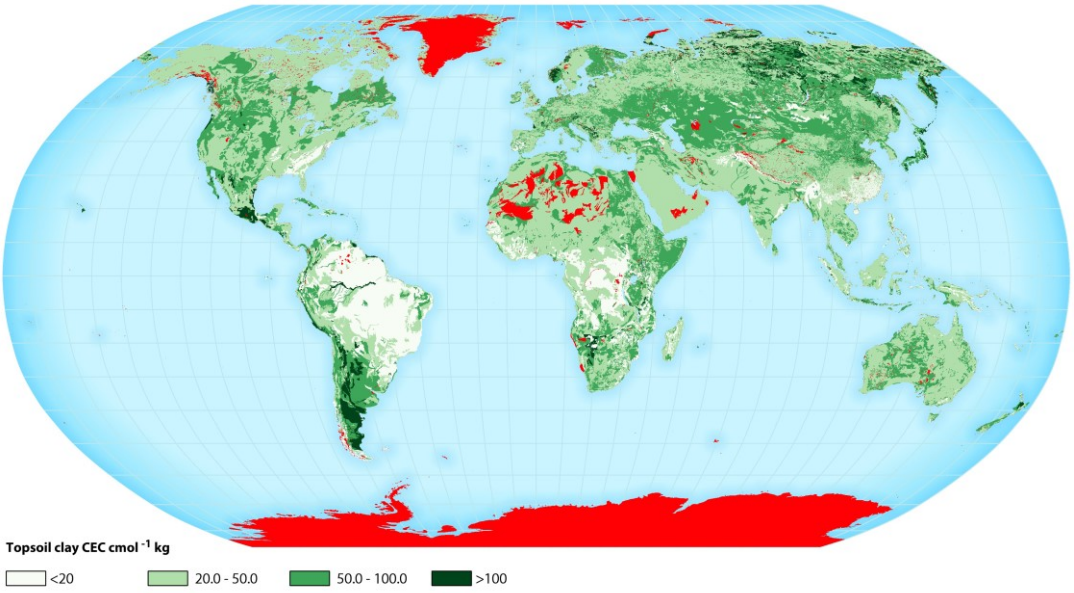


Figure 1.11 - Global distribution of the (a) clay content (% weight) and (b) clay cation exchange capacity (CEC; cmol-1 kg clay) in the top 30 cm of soil (FAO/IIASA/ISRIC/ISS-CAS/JRC 2009).

Clays generally have a cation exchange capacity (CEC) at pH values found in terrestrial systems because of isomorphous substitution (i.e. the replacement in the mineral structure of one cation with another of a different charge) and de-protonation of

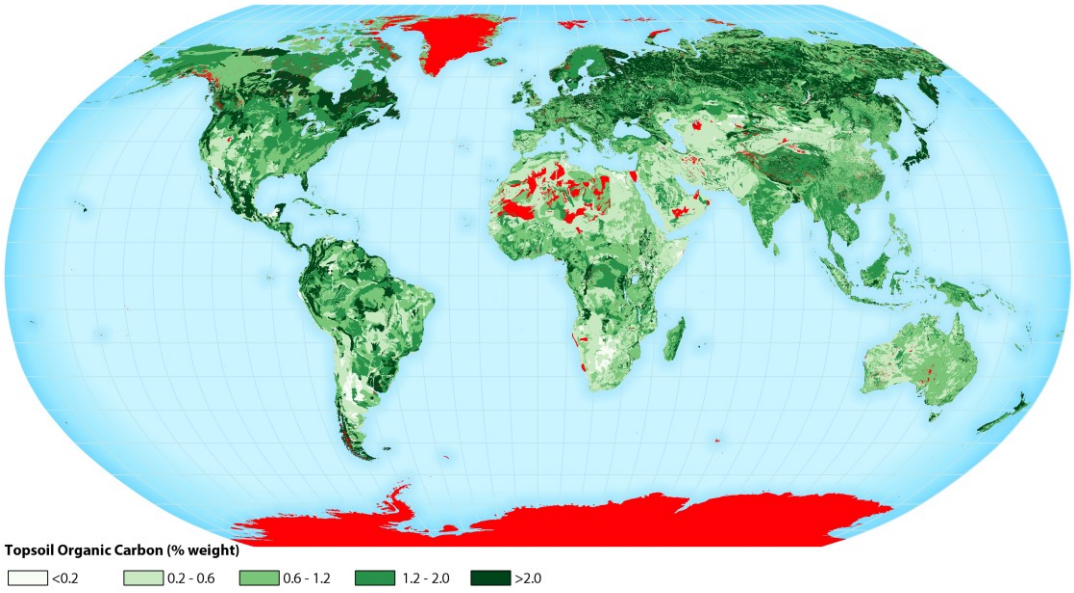
surface hydroxyl groups (the extent dependent on pore water pH) while the overall magnitude of the CEC value will depend on the type of clay. The higher the CEC value the more sorption sites are potentially available. The distribution of the clay CEC in surface soils across the globe varies considerably (Figure 1.11(b)) (FAO/IIASA/ISRIC/ISS-CAS/JRC 2009). Clay CEC values are generally in the range 22-55 $cmol^{-1}$ kg clay (at pH 7) across many of the LLMIC of interest. Values are lower in south eastern China and central South America (< 22 $cmol^{-1}$ kg clay, and often < 10 $cmol^{-1}$ kg clay), and higher in areas of north and western China, central India, the coastal zones of north Africa and western South America. Clays in Mexican soils have some of the highest values (> 104 $cmol^{-1}$ kg clay) observed. In relatively acidic environments however, clays may exhibit an anion exchange capacity (AEC) because of protonation of the surface hydroxyl groups. The AEC:CEC ratio will reflect the difference between the soil pore water pH and the pH of the net zero charge of the particular clay type (Hyun et al. 2004). Thus, ionic sorption of an API to the clay will be a function of the AEC:CEC ratio, soil solution pH and API pK_a. Clay CEC has been reported to be important for the sorption of some antibiotic APIs (Vasudevan et al. 2009; Yan et al. 2012).

1.3.1.7 Soil organic matter

Organic matter is a heterogeneous mixture of organic compounds with varying characteristics that depend on the source of the constituent materials (Park et al. 2018). Soil organic matter, which represents the variable decomposition products of autochthonous and allochthonous inputs of organic matter (animal, plant, microbial biomass) to soils, is comprised of particulate organic matter (POM) and, in pore water, colloidal dissolved organic matter (CDOM). Figure 1.12(a) shows the global distribution of POM, as organic carbon, in surface soils (FAO/IIASA/ISRIC/ISS-CAS/JRC 2009) and most LLMIC that use wastewater for irrigation (Figure 1.4a), have soils with organic

carbon concentrations above 0.6 % (w/w), indicating fertile soils with, in principle, good contaminant sorption properties. In contrast, soils in northern China, Pakistan and much of North Africa are low in organic carbon (< 0.6 % w/w) and probably need fertiliser to be productive, so wastewater irrigation would likely enhance the organic carbon content of these soils (Qadir et al. 2010).

(a)



(b)

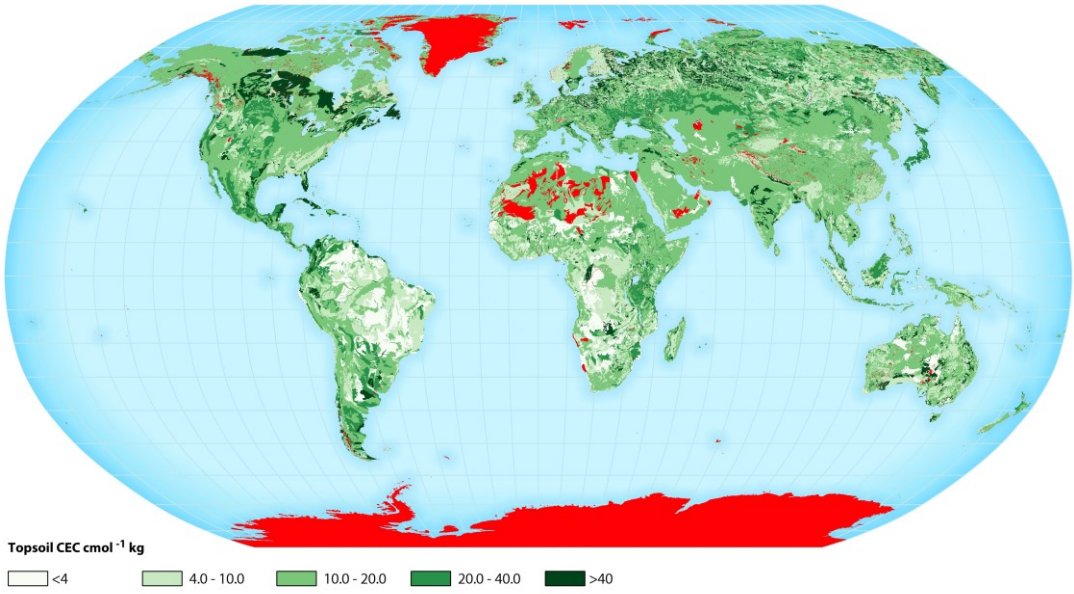


Figure 1.12 - Global distribution of the (a) organic carbon (% weight) and (b) soil cation exchange capacity (CEC; cmol-1 kg soil) in the top 30 cm of soil (FAO/IIASA/ISRIC/ISS-CAS/JRC 2009). Coverage in red indicates no data.

Important fractions of soil POM occur as polymeric macro-molecules with relatively high aromatic and alkyl content (Kleber et al. 2007). Partitioning of hydrophobic contaminants into this fraction has historically been quantified empirically by the organic carbon–water partition coefficient, K_{oc} (ECETOC 2013a), and APIs with aromatic constituents and/or high carbon fraction may partition into the hydrophobic POM fraction and contribute to the API K_{oc} value. A range of APIs (carbamazepine, ibuprofen, naproxen and diclofenac, for example) were generally better retained in soils with relatively high POM and their concentrations were positively correlated with POM concentrations {Chefetz, 2008 #26;Drillia, 2005 #76;Gibson, 2010 #49;Xu, 2009 #135. In contrast, partitioning into soil solids of these APIs was less extensive in low POM soils because of reduced hydrophobic interactions with the POM and probable binding of API with CDOM in some cases.

The sorption of neutral APIs by soil organic matter is well correlated and dependent on the characteristics of the organic matter {Park, 2018 #556;Huang, 2003 #559}. Soil organic matter is composed of many different organic materials ranging from biopolymers (e.g. polysaccharides, lipids, proteins and lignin), humic substances derived from biopolymers and diagenetically matured kerogen and combustion-related black carbon or char materials (Aiken et al. 1985; Huang et al. 2003). The different combination of these organic materials will impact the rate and strength of sorption of any organic compound to the soil organic matter (Huang et al. 2003). Cavities within the soil organic matter structure have also been shown to have a large effect on both hydrophilic and hydrophobic pollutants in soils, the strength of this effect is dominated by the size of the cavity, with smaller cavities having greater binding energies involved to neutral and charged organic molecules (Ahmed et al. 2015).

Carbamazepine, a neutral API, has been speculated to exhibit stronger sorption interactions with hydrophobic rather than polar soil organic matter (Chefetz et al. 2008). More polar organic matter is usually found in the top 0-5 cm of agricultural fields due to the presence of partially decomposed relatively polar organic materials, whereas at depth the decomposition is greater reducing the quantity of these partially degraded sorbents (Chefetz et al. 2000). This has been identified in soils taken from different depth down a soil profile exhibiting different sorption affinities for carbamazepine. The upper layer had less sorption of the API compared to lower depths (Chefetz et al. 2008). However a different study found the removal of carbamazepine from two soils with differing hydrophobicity, size of organic molecules and electrical charge densities was not affected by differences in soil organic matter (Park et al. 2018).

POM has a CEC because of a pH-dependent net negative surface charge. CEC values of 60 - 300 cmol kg^{-1} organic carbon (at pH 7) may account for 25 - 90% of the total CEC of soils, and in some cases may be more important than clays. Thus transfer of the neutral and cationic forms of all but the most water-soluble APIs from solution to POM will occur (Kinney et al. 2006; Gibson et al. 2010) and it can be concluded that the K_{oc} value will comprise both a hydrophobic and a hydrophilic component. Figure 1.12(b) shows the global distribution of top soil CEC due to both organic matter and clay (FAO/IIASA/ISRIC/ISS-CAS/JRC 2009). Soils with CEC values below 10 $cmol^{-1}$ kg soil are considered poor at cation retention, and these occur in south eastern, northern and western China, coastal regions of India, confined areas of North Africa and much of South America. Elsewhere, intermediate (22-55 $cmol^{-1}$ kg soil) values of CEC dominate, with highest values found in parts of central India and throughout Mexico.

In both high and low POM soils, the transport of APIs may be enhanced by irrigation with treated and untreated wastewater (Drillia et al. 2005; Gibson et al. 2010). Thus, the formation of API–CDOM complexes, as discussed below, may contribute to

enhanced API solubility and mobility if the CDOM is not surface reactive, but API mobility may decrease if surface sorption occurs (Chefetz et al. 2008; Blackwell et al. 2009). Like POM, CDOM will be comprised of a humic-like hydrophobic fraction and a water-soluble, more polar, component. CDOM generally carries a pH-dependent net negative surface charge due to ionised carboxylic acid and phenolic groups, and the more polar constituents are more abundant at the lower end (< 1 kDa) of the CDOM size range (Yang et al. 2011).

The ability of surface water CDOM or its humic components, to bind organic contaminants has been reported since the 1980s (Chiou et al. 1986; Chiou et al. 1987; Yang et al. 2011), although to date there have been very few mechanistic studies of this phenomenon for APIs and wastewater or soil CDOM (Blackwell et al. 2009). The antibiotic ciprofloxacin was reported to partition into humic material CDOM to a much greater extent than into CDOM present in treated municipal wastewater (Carmosini et al. 2009). The mechanism of sorption to the humic CDOM was pH-dependent cation exchange. Similar partitioning to the polar compound rich wastewater CDOM was not observed because of the relatively high alkalinity of the wastewater. For a range of surface waters (river, estuary) the binding of APIs to CDOM appeared to be dominated by the larger (> 1 kDa) hydrophobic fractions, although the pH dependency of this phenomenon was not explored (Liu et al. 2005; Maskaoui et al. 2007; Yang et al. 2011). The partitioning of the APIs into the estuarine CDOM was 2–4 orders of magnitude higher than into (OC–normalised) suspended matter, emphasising the importance of CDOM to API binding in surface waters (Yang et al. 2011). It has been reported that the character of CDOM (e.g. hydrophobic/hydrophilic balance, molecular size/weight distribution) can change during wastewater treatment, and with type of treatment (Shon et al. 2006). It is likely, therefore, that the binding and reactivity of an API in untreated and treated wastewater may be quite different and perhaps impact on its fate in irrigated soils (Gibson

et al. 2010). From these findings it would appear that studies of API–wastewater or API-soil organic matter interactions should become a focus for research.

1.3.1.8 Clay – organic matter interactions and API sorption

While the clay and soil organic matter constituents have been described separately in order to highlight the global variations in their physico-chemical characteristics, in reality these moieties exist as intimate, and complex, organo-mineral assemblages as a result of solid – solution interactions; indeed, in temperate soils, 50 – 75 % of the soil organic matter is assemblage material (Christensen 2001). In the last two decades an arguably realistic conceptual model of these assemblages has emerged (Kleber et al. 2007). This zonal model, in principle, allows for the chemical bonding mechanisms between APIs and soil components (Figure 1.8) that are understood to occur (ECETOC 2013b). These mechanisms include van der Waals, hydrogen and covalent bonding, ionic and ligand exchange, charge transfer, hydrophobic interactions and cationic bridging (Figure 1.8). The model also allows for physical sequestration into the soil organic matter matrix. Empirical data are beginning to reveal that the assemblages occur as discrete clusters and that they are the primary sites for interaction with organic matter in soil pore water. Furthermore, most (ca. 80 %) of the clay mineral surface, which has been generally perceived as reactive to OM, does not interact with the pore water organic matter at all (Vogel et al. 2014). If these findings are corroborated, it may serve to further increase the difficulty in predicting API sorption and fate, and subsequent development of ERAs for LLMIC soils.

1.3.2 Modelling approaches to soil sorption

When experimental data is not available soil sorption modelling can provide a useful insight for understanding the potential fate of APIs (Droge et al. 2013). There are several studies that have developed predictive models for estimating soil sorption of APIs (Franco et al. 2008; Barron et al. 2009; Droge et al. 2013).

Barron et al., (2009) used artificial neural networks, a non-linear correlation modelling technique, to predict K_d in soils and sewage sludge. This type of modelling can be used to find correlations in complex data sets and was inspired by the functioning of human brains, using interconnected neurons to process information in parallel that are capable of learning by adjusting the statistical weight on connections to minimise the overall or absolute error (Wang 2003; Barron et al. 2009). This technique can only provide predictions based on the data sets used for training. Barron et al., (2009) used 37 inputs into the model relating to the structure of the API, including, molecular weight, $pK_{a,}$ number of rotatable bonds etc. Artificial neural networks are complex models to run and usually require specialist computers along with a large reliable training dataset (Marini et al. 2008).

Droge and Goss (2013) developed a statistical model for sorption of organic cations in soil. This model sums the contribution organic matter and phyllosilicate that clay minerals provide to cation sorption. In this model, sorption to organic matter is normalised to the fraction of organic carbon and sorption to clay is normalised to the estimated CEC attributed to clay minerals. The approach of separating cation exchange between the two main soil sorption sites and representing structural feature contributions of those sites has been shown to be successful for predicting sorption of organic cations to two soils and added an advance in the previous models for cations (Jolin et al. 2017).

Franco and Trapp (2008) developed a semi-empirical model that predicts K_{oc} for acids, bases and amphoteric organic compounds. The hydrophobic sorption of the neutral fraction of an API is separated from the sorption associated with the ionic fraction (ECETOC 2013a). It uses Log K_{ow} and pK_a to predict the K_{oc} of an ionisable API (Franco et al. 2008). Franco and Trapp (2008) identified some limitations within the model. These include; clay content of soils was not taken into account and variations of soil away from a normal ratio of organic carbon to clay and complexation of organic ions with metals

and ligands of opposite charges is not included. Due to a lack of available computing power and time constraints the research in this thesis required an efficient model that could provide good estimates of K_{oc} over a range of pK_a values. The Franco and Trapp (2008) model was chosen to predict the K_{oc} in Chapter 4 of this thesis due to small computational footprint and use in literature (Paszko 2012; Schaffer et al. 2012; Al-Khazrajy et al. 2016).

1.4 Environmental risk from APIs in soils of LLMIC

A 'risk' is defined as the measure of the probability that harm will occur after exposure to a chemical (Duffus et al. 2001). Risk assessment is the procedure in which the risks posed by hazards are estimated depending on the environmental exposure (Fairman et al. 2008).

In Europe, an API is considered to be a risk to the environment and subject to further testing when its Log K_{ow} is > 4.5, when the surface water PEC exceeds 0.01 µg L^{-1} (lower for compounds with mode of action related concerns), when the surface water PEC:PNEC ratio is > 1, or when the surface water PEC:PNEC (microorganism) ratio is > 0.1 (EMEA 2006). Confidence in this type of assessment is related to the data available and in some cases assumptions are required including worst case scenarios and read across from other environmental compartments. For example, EMEA (2006) does not require the calculation PECs and PNECs in soil for APIs with a Log K_{oc} <4. The soil PNECs reported in Figure 1.13 for a range of APIs are predicted from the surface water PNEC (black crosses) using the partition coefficient of the API in soil and the bulk density of wet soils (Table 1.3)(ECB 2002). Soil PNECS that have been estimated in this manner may not stand up to critical assessment as the aquatic organisms used to determine PNECs in surface water have different ecotoxicology endpoints, exposure pathways and sensitivities compared to terrestrial organisms (Bourdat-Deschamps et al. 2017). Data

was available in literature for five of the APIs in Figure 1.13 (black triangles).These calculations reveal that measured soil concentrations is higher than the PNECs for ciprofloxacin, fluoxetine, ofloxacin, oxytetracycline, triclosan and tetracycline in some cases (Figure 1.13). In these cases the risks to terrestrial organisms may be having a negative impact on the soil fauna health.

Although soil concentrations of APIs in LLMIC are generally low, the dataset is restricted both spatially and temporally, as shown in Table 1.2, and so there is uncertainty regarding risk to humans and other biota (Qin et al. 2015). Furthermore, while PNECs are formulated for acute toxicity assessments, chronic effects may be more important, particularly as wastewater irrigation becomes more widespread.

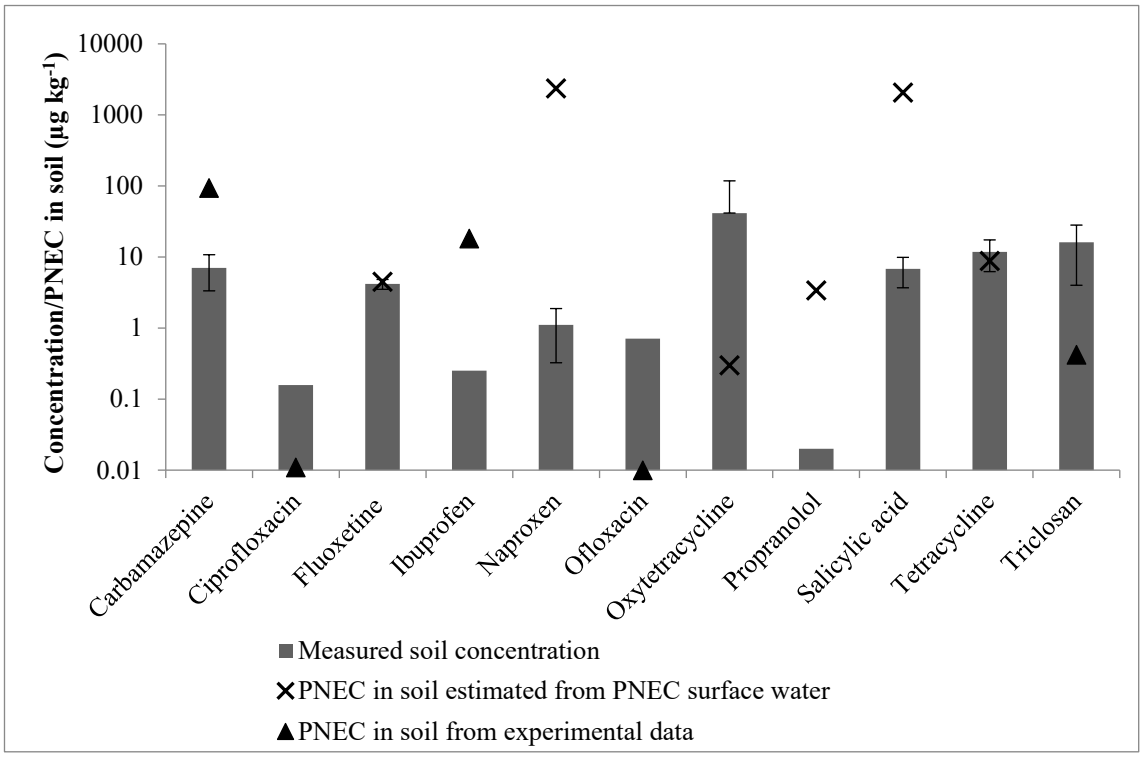


Figure 1.13 - Concentrations of selected APIs in global soils with the corresponding estimated PNEC in soil. Error bars represent ± standard deviation (n = 3-8). (Jones et al. 2002; Durán-Alvarez et al. 2009; Gibson et al. 2010; Oakes et al. 2010; Wu et al. 2010; Zhao et al. 2010; Chen et al. 2011; Li et al. 2011; AstraZeneca 2012; Martín et al. 2012; Vazquez-Roig et al. 2012; Rutgersson et al. 2014; Bourdat-Deschamps et al. 2017)

Table 1.3 - API risk assessment data

API	Treatment Area	% dose excreted	Aquatic PEC (µg L^{-1})	Aquatic PNEC (µg L^{-1})	Aquatic PEC/ PNEC	Soil PNEC[b] (µg kg^{-1})	pKa	Log K_{oc} (L kg^{-1})	Log K_{ow}	K_d soil[b] (L kg^{-1})	K_d sewage sludge[b] (L kg^{-1})	Water solubility (g L^{-1})
Atenolol	Cardiovascular		0.68	148	0.0046	1306	9.6	2.6	0.015	7.96	2.95	26.5
Propranolol	Cardiovascular	17	0.044	0.1	0.44	3.4	9.53	3.21[k]	-0.12-2.6	58[k]	600.07	97.9
Ciprofloxacin	Infection	84[c]	0.035[g]	991[g]	0.0035	248915	6.09[g]	3.1[h]	0.28[i]	427[h]	417[h]	30[i]
Norfloxacin	Infection	50-68[p]	0.24[q]	40[q]	0.006	47-12611	6.30, 8.38[p]	4.8-5.5[p]	-1.03[q]	2-536[r]	23345.42 - 117004.3	0.35[p]
Ofloxacin	Infection		0.05[g]	3.13[g]	0.02	569	6.05, 8.51[g]	4.6[h]	-0.39[i]	309[h]	1.7[h]	28.26[i]
Oxytetracycline	Infection		0.83[d]	0.23[d]	3.6[d]	0.3	3.3, 9.1[j]	1.99[d]	-0.9[i]	1.95	36.16	0.313[i]
Triclosan	Infection			0.05[r]		3.74	7.8[e]	3.54[k]	4.8[e]	127[k]	1283[k]	0.005[i]
Carbamazepine	Neuroscience	1-2[c]	1.23[d]	6.36[d]	0.19[d]	48.6	7[e]	2.56	2.45[i]	5.64	2.09	0.112[i]
Diclofenac	Neuroscience	15[c]	0.8[d]	138.74[d]	0.0058[d]	735	4.15[j]	2.39[k]	4.51[i]	9[k]	105[k]	0.0024[i]
Fluoxetine	Neuroscience	3-11[c]	0.052[l]	0.012[l]	4.3[l]	1.8-4.5	10.06[l]	4.1-4.5[l]	4.26[l]	251.79-632.46	4658-11700	60.3[l]
Ibuprofen	Neuroscience	1-8[c]	4.96[d]	9.06[d]	0.55[d]	3.0	4.91[e]	2.01-2.11[m]	3.97[i]	0.56-3.71[m]	37.86-47.67	0.021[i]
Naproxen	Neuroscience	0-10[n]		21.2[o]		2357	4.2[d]	2.48[k]	3.18[i]	11[k]	36[k]	0.016[i]

[a] Data with no citation are from AstraZeneca Environmental Risk Assessment Data (AstraZeneca 2012). [b] Soil PNEC, K_d soil and K_d sewage sludge were predicted using the TGD method unless stated otherwise (ECB 2002). [c] (Jjemba 2006). [d] (Jones et al. 2002). [e] (Azzouz et al. 2012). [f] (Vazquez-Roig et al. 2012). [g] (NCCOS 2006). [h] (Thiele-Bruhn 2003).[i] (Chen et al. 2011). [j] (Berthod et al. 2014). [k] (Barron et al. 2009). [l] (Oakes et al. 2010). [m] (Xu et al. 2009a). [n] (Carballa et al. 2008). [o] (Martín et al. 2012). [p] (Peruchi et al. 2015). [q] (Perazzolo et al. 2010). [r] {Chen, 2014 #231

Estimates of annual API loading to soils from wastewater irrigation may be obtained by using representative irrigation rates and measured concentrations of APIs in irrigation waters from LLMIC. While this type of calculation has been performed for high income countries {Qin, 2015 #202}, it is rare for LLMIC because data on API concentrations in irrigation water are not readily measured and/or available. In principle, the resulting maximum concentrations in soils can then be calculated, assuming zero losses (i.e. from lateral run-off, loss to groundwater, biotransformation) and representative infiltration depths and soil densities. Table 1.4 shows results using this approach for soils in Tula Valley (Mexico), where extensive irrigation using untreated wastewater occurs (Gibson et al. 2010). The calculated maximum soil API concentrations for naproxen, diclofenac and carbamazepine are significantly lower (> order of magnitude) than the predicted no effect concentrations (PNEC) for soil of 2357 $\mu g\ kg^{-1}$, 735 $\mu g\ kg^{-1}$ and 48.6 $\mu g\ kg^{-1}$, respectively (PNEC values are reported in Table 1.3). In contrast, the PNECs for ibuprofen and triclosan, of 3.0 $\mu g\ kg^{-1}$ and 3.74 $\mu g\ kg^{-1}$, respectively, are within an order of magnitude of the calculated maximum soil concentrations. Coupled with data on sorption of APIs to soils and API persistence, this type of analysis is essential for the estimation of predicted environmental concentrations (PECs) of APIs, which are central to the development of robust environmental risk assessments.

Table 1.4 - API loads to soils in Tula Valley, Mexico, from untreated irrigation wastewater and estimated maximum soil concentrations* (Gibson et al. 2010)

API	API concentration ($\mu g\ L^{-1}$)	API annual load (μg)	Max soil API concentration ($\mu g\ kg^{-1}$ DW[a])
Naproxen	7.3	2190	4.87
Naproxen	13.6	4080	9.07
Ibuprofen	0.74	222	0.49
Ibuprofen	1.41	423	0.94
Diclofenac	2.05	615	1.37
Diclofenac	4.82	1446	3.21
Carbamazepine	0.084	25.2	0.06
Carbamazepine	0.24	72	0.16
Triclosan	0.084	25.2	0.06
Triclosan	1.03	309	0.69

[a]DW – dry weight of soil

* Irrigation rate – 300 L water $m^{-2}\ y^{-1}$, infiltration depth 0.3 m, soil density 1500 kg m^{-3}, soil mass 450 kg (ECB 2002)

The toxicity of APIs to non-target organisms will depend on, *inter alia*, the speciation of the compound (i.e. the relative amounts of free and bound chemical and their lipophilicities). The amelioration of xenobiotic compound toxicity due to binding with polymeric aromatic humic and fulvic acids has been reported since the 1980s (Chiou et al. 1986; Chiou et al. 1987; Oris et al. 1990; Day 1991; Haitzer et al. 1998). For example, fulvic and protein rich CDOM in wastewater effluent reduced endocrine disrupting chemical toxicity to biota; the most effective CDOM was in the < 0.2 µm size fraction, while CDOM in the < 5 kDa fraction did not reduce toxicity of the endocrine disrupting chemical (Lee et al. 2011). The influence of CDOM on the speciation, bioavailability and toxicity of metals in surface waters is now well established (Aiken et

al. 2011). The resulting Biotic Ligand Model of CDOM–metal interactions represented a major advance in metal toxicity standards and is now enshrined in EU and USA environmental quality standards (Comber et al. 2008; Aiken et al. 2011). Similar toxicity amelioration experiments of wastewater CDOM or soil solids with APIs have not been reported (Qin et al. 2015). Following the experience with metals, this is an area of study that clearly merits further effort from the scientific and regulatory communities.

In summary, there is a paucity of information on the potential ecotoxicological impacts of APIs in the terrestrial environments of LLMIC (Kookana et al. 2014). While knowledge transfer from high income countries to LLMICs of API soil biogeochemistry will prove of benefit in some instances, basic datasets on API loadings to the environment, coupled to more systematic measurements of free and bound APIs in soils and waters, are needed so that realistic PECs can be calculated and resultant exposures of biota to contaminant pharmaceuticals elucidated. The development of any subsequent risk assessments, equivalent to the Phase II effects testing of EMEA (2006), should use appropriate test organisms.

1.5 Conclusions

Water scarcity in LLMICs and the increasing use of APIs globally has led to concerns about the input of APIs and other down the drain chemicals to soils during irrigation with wastewater, a concern that has now been recognised by the International Conference on Chemical Management. Wastewater reuse for irrigation is currently not included in terrestrial environmental risk assessments of APIs and terrestrial assessments are only conducted for APIs with a Log K_{oc} >4, in Europe or elsewhere. For the development of API risk assessment-type frameworks within LLMIC there remains much to do. The datasets missing include accurate API usage in LLMICs, estimates of point

and diffuse sources of APIs, (including removal efficiencies during effluent processing in sewage treatment plants, where this occurs), to soils and waters, sludge and irrigation water application rates to land, and the speciation and partitioning of the APIs in those compartments. Soil physico-chemical factors and the chemical structure and behaviour of APIs will influence their fate in soil. A wide range of soil conditions exist in LLMIC globally, making the development of predictive models of soil behaviour, distributions and fate of APIs a challenge. For more extensive assessments of API behaviour and effects, where required, within an environmental risk assessment framework, it is a prerequisite that methods are appropriate. For example, while OECD methods 106 and 307 for determining the fate of ionisable chemicals in soils largely cover the range of soil properties (pH, clay content, soil organic matter concentration) to be found in LLMIC, care must be taken in the selection of soils used for testing to ensure that they are representative for the region of interest.

The development of environmental risk assessments is a resource intensive process. However, without robust monitoring in combination with mechanistic fate data for the partitioning and degradation of APIs in soils of LLMIC countries, it is not possible to address the risk of APIs in the environment. The limited data available for APIs in soil identified here, suggests that within LLMIC, regulators, the wastewater treatment industry, the relevant pharmaceutical sectors, and other stakeholders should co-operate in the development of ERAs in the most cost-effective way.

1.6 Research aim and objectives

1.6.1 Research aim

Following on from this literature review the overall aim of this thesis was to investigate how wastewater input to soil affects the sorption fate of APIs in soils and how standard experiments may need to be improved to include this input of APIs to the soil environment. Data from these experiments might be able to suggest improvements to the current terrestrial environmental risk assessments.

1.6.2 Research objectives

The specific research objectives of this thesis that were achieved were:

1. Review current literature regarding the occurrence and sorption fate of APIs in soils in LLMICs and soil properties that influence this (Chapter 1)
2. Develop a robust analytical method on HPLC-HRAM-MS that allows for the analysis of four APIs in one injection to reduce time required on the instrument. Describe and use other methods to support this research (Chapter 2)
3. Investigate soil sterilisation methods that are commonly used to distinguish biodegradation and sorption studies and identify which method might be best to use (Chapter 3)
4. Study the sorption and desorption fate of APIs in two soil matrices and identify differences between the two soils (Chapter 4)
5. Study the effect the addition of a synthetic wastewater to soil matrices on the sorption fate of APIs during the OECD 106 sorption experiment (Chapter 5)
6. Link changes in soil properties from wastewater irrigation to fate of APIs in LLMICs using wastewater irrigation and how current ERAs could be developed further to include this source of APIs to soils (Chapter 6)

At the beginning of this research, the following additional objectives were set but were not achieved due to experimental limitations:

- Investigate how variations in wastewater quality impacts the sorption fate of APIs in soils (e.g. variations in pH, organic carbon, nutrients and microorganisms)
- Complete column dissipation studies to provide data on the movement of APIs through soils and how wastewater irrigation affect this. This experiment would include a number of columns with various irrigation schemes to compare the volume and characteristics of wastewater irrigation cycles to regular rainfall events
- Use soils collected from LLMICs in the sorption experiments to understand how soils from regions that use wastewater irrigation differ from those in Europe (Lufa Speyer soils)

2 Method development and standard soil methodologies

2.1 Introduction

The aims and objectives for this research were achieved through a range of laboratory experiments. This chapter outlines method development, starting with the selection of APIs, soils and apparatus used. Details of HPLC-MS analytical method development is then described. Finally, standard soil methods are described including pH, assessment of overall soil charge, dissolved organic carbon and the identification of humic and fulvic soil components. All of the laboratory work in this chapter was completed at the University of Plymouth. Methods specific to an experiment are outlined in the relevant chapter.

2.2 API selection

Three APIs were initially chosen for this research: propranolol, naproxen and ofloxacin. These three initial APIs were chosen after an extensive literature search had been completed. This identified measured pK_a, Log K_{oc}, Log K_{ow}, and these APIs were likely to be able to be analysed by HPLC-HRAM-MS (Table 1.3). It was important that there was some previous data already available for these APIs to ensure that the sorption in soil experiments were completed successfully before adding synthetic wastewater in Chapter 5. A fourth API, nevirapine, was added at a later stage of this project to provide wider information on the fate of APIs in soils and the impact wastewater irrigation has on their fate. This API has had no sorption experiments in soil that could be found in literature so the data collected in this chapter was novel. Additionally these APIs were selected on the basis that they are used widely in areas where wastewater irrigation occurs, while covering a wide range of physico-chemical properties, particularly the charge associated with the molecule at the pH range encountered in the experiments and they cover a wide range of therapeutic classes. This research was limited to four APIs due to analytical time constraints, both for method development and running samples.

2.2.1 Ofloxacin

Ofloxacin (Figure 2.1) is a fluoroquinolone antibiotic used to treat infections of the respiratory tract, kidney, skin, soft tissue and urinary tract. It is a broad spectrum antibiotic and is active against Gram-positive and Gram-negative bacteria (DrugBank 4.3 2013b). The defined daily dose (DDD)[2] is 0.4 g via the oral and parenteral (e.g. by injection or intravenously) methods of administration (WHO 2015b). Excretion occurs mainly via urine at 70 - 98 % as the parent compound, while small amounts of desmethyl ofloxacin and ofloxacin N-oxide have been detected in some studies (Monk et al. 1987).

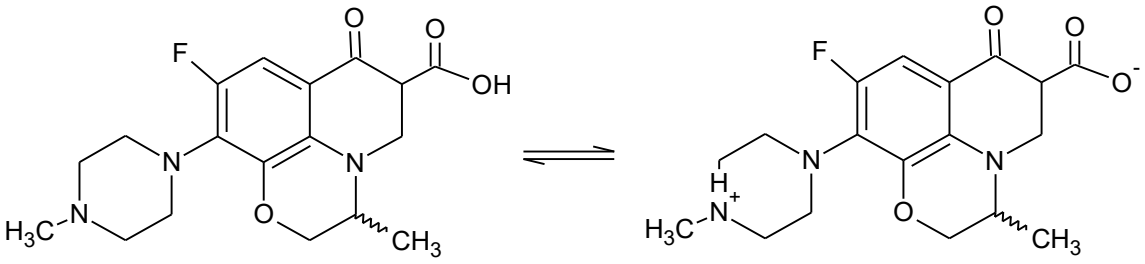


Figure 2.1 - Structure of ofloxacin (left) and its zwitterion

Ofloxacin was selected for study due to its hydrophobic nature, low wastewater treatment plant (WWTP) removal rate (Table 2.1 and Table 2.2) and broad-spectrum use (i.e. as both human and veterinary medicine). Prescription rates of antibiotics in industrialising countries are difficult to locate as there are no official records of use (Leung et al. 2012). In a study surveying antibiotic use in private retail pharmacies, public sector facilities and private clinics of New Delhi, India, ofloxacin was the most widely used fluoroquinolone at 43.9, 41.5 and 48.9 % respectively (Kotwani et al. 2011). In China, ofloxacin is the most commonly used fluoroquinolone at 39.0 % (1286 tons in 2013) in human medicine; it is also commonly used in pig and chicken production (2449

[2] DDD is the assumed average maintenance dose per day for a drug used for its main indication in adults WHO. 2009. Ddd definition and general consideration. Available: http://www.whocc.no/ddd/definition_and_general_considera/ [accessed 01 June 2017].

and 832 tonnes, respectively, in 2013) (Zhang et al. 2015). Ofloxacin has a Log K_{oc} > 4, triggering the need for an extended terrestrial risk assessment to be undertaken (Table 2.2).

Table 2.1- Properties of ofloxacin

Formula	$C_{18}H_{20}FN_3O_4$	
CAS Number	82419-36-1	
Purchase information	≥ 99%, Sigma Aldrich	
Monoisotopic mass	361.143784 Da	
$[M+H]^+$	362.151061 Da	
$[M-H]^-$	360.136508 Da	
Melting point	225 °C (experimental)	(ChemSpider 2015c)
Water solubility	25 mg mL^{-1} (experimental)	(ChemSpider 2015c)
pK_a	5.97, 8.28	(Drillia et al. 2005)
$LogK_{ow}$	-0.39 (experimental)	(U.S. National Library of Medicine)

Table 2.2 - Environmental parameters of ofloxacin

Property		Comments	Ref
WWTP removal	57 %	Italy, primary settling and activated sludge processes	(Castiglioni et al. 2005)
WWTP removal	< 12 %	Hong Kong, China, settling and activated sludge processes	(Leung et al. 2012)
WWTP removal	60 %	Average of 11 activated sludge WWTPs in UK	(Gardner et al. 2013)
WWTP removal	49 %	Average of 12 fixed film WWTPs in UK	(Gardner et al. 2013)
K_d soil	309	pH 5.3, TOC 0.7%, CEC 12 $cmol^{-1}$ kg, 2.5% clay	(Nowara et al. 1997)
K_d soil	3554	pH 4.3, TOC 7.1%, 15.84% clay	(Drillia et al. 2005)
K_d soil	1192	pH 6.8, TOC 0.37%, 43.28% clay	(Drillia et al. 2005)
Log K_{oc}	4.64	pH 5.3, TOC 0.7%, CEC 12 $cmol^{-1}$ kg, 2.5% clay	(Nowara et al. 1997)
Log K_{oc}	5.70	pH 4.3, TOC 7.1%, 15.84% clay	(Drillia et al. 2005)
Log K_{oc}	5.51	pH 6.8, TOC 0.37%, 43.28% clay	(Drillia et al. 2005)
Half-life in soil	1386	pH 5.6, TOC 1.7 %, 20% clay, 27% silt, 53% sand	(Walters et al. 2010)
Half-life in soil	750-1500	pH 7.1, OC 13.7 g kg^{-1}, CEC 9.8 $cmol^{-1}$ kg	(Bourdat-Deschamps et al. 2017)
Half-life in soil	<110	pH 6.1, OC 21.4 g kg^{-1}, CEC 10.6 $cmol^{-1}$ kg	(Bourdat-Deschamps et al. 2017)
PEC	50 ng L^{-1}		(Vazquez-Roig et al. 2012)

*PEC – Predicted environmental concentration, TOC – total organic carbon, CEC – cation exchange capacity

2.2.2 Propranolol

Propranolol is a beta-blocker used globally for the treatment of hypertension, angina, anxiety and prevention of migraine (AstraZeneca 2012) (Figure 2.2 and Table 2.3). The DDD is 0.16 g in the oral and parenteral methods of administration (WHO 2015c). It is completely metabolised in the body and 95 - 100 % of the dose is excreted as metabolites and their conjugates in urine (AstraZeneca 1997). Conjugated propranolol (chloride form) accounts for 17 % of the excreted dose, which can potentially dissociate to uncomplexed propranolol during sewage treatment (AstraZeneca 2012; Sun et al. 2014).

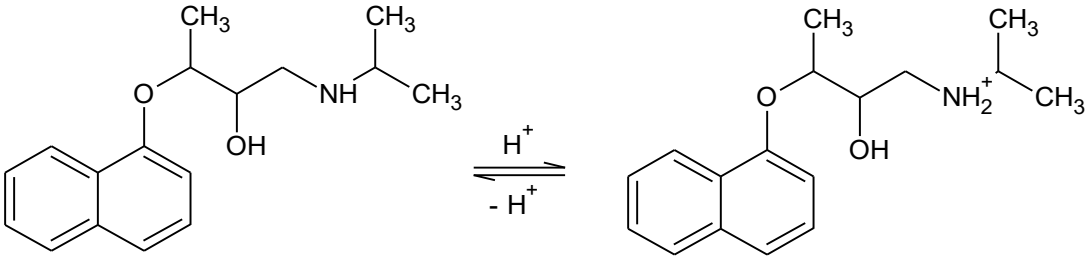


Figure 2.2 - Structure of propranolol and its cation. It was purchased in solid form as its hydrochloride.

Propranolol was chosen due to its wide usage globally and variable global WWTP removal rates (-10 to 90 %), along with the fact that its log K_{oc} value of 3.21 - 4.69 is below the trigger of 4 in some soils and above in others and it has a positive charge at environmental pH (Table 2.4) (EMEA 2006). Net gain of APIs in WWTPs (Table 2.4) can be caused by poor removal rates and API conjugates entering the WWTP being cleaved during processing to give an apparent gain in parent compound (Sun et al. 2014). It has been studied in several environmental compartments, including soil, aiding the development of laboratory methods and understanding that can then be transferred to less studied APIs.

Table 2.3 - Properties of propranolol

Formula	$C_{16}H_{21}NO_2$	
CAS	318-98-9	
Purchase information	≥99% purity, Sigma Aldrich	
Monoisotopic mass*	259.157229 Da	
$[M+H]^{+*}$	260.164505 Da	
$[M-H]^{-*}$	258.149952 Da	
Melting point	163-166 °C (experimental)	(ChemSpider 2015d)
Water solubility	6 g L^{-1} (experimental)	(ChemSpider 2015d)
pK_a	9.53	(Carter et al. 2014b)
$LogK_{ow}$	3.48 (experimental)	(U.S. National Library of Medicine)

Table 2.4 - Environmental parameters of propranolol

Property		Comments	Reference
WWTP removal	32 %	Sweden, bar screening, grit removal, primary clarification, activated sludge, secondary sedimentation, chemical phosphorous removal and final sedimentation	(Bendz et al. 2005)
WWTP removal	65 %	Germany, primary clarifier, two stage anaerobic digester	(Ternes et al. 2007)
WWTP removal	-10 % (net gain)	China, primary treatment process, Orbal oxidation ditch process and UV disinfection process	(Sun et al. 2014)
WWTP removal	-10 – 90 %	5 WWTPs in India all using activated biological treatment	(Subedi et al. 2015)
K_d soil	199 ± 9.6	pH 4.3, TOC 7.1 %, 15.84 % clay	(Drillia et al. 2005)
K_d soil	16.3 ± 1.4	pH 6.8, TOC 0.37, 43.28 % clay	(Drillia et al. 2005)
K_d soil	1100	pH 6.6, TOC 2.2 %, CEC 28 $cmol^{-1}$ kg	(Yamamoto et al. 2009)
K_d soil	58	pH 6.3, 3.77 % TOC, 20 % clay	(Barron et al. 2009)
K_d soil	154.06 ± 6.95	pH 6.22, OC 14.9 %, 7.2 % clay, peat soil	(Maszkowska et al. 2014)
Log K_{oc}	3.45	pH 4.3, TOC 7.1%, 15.84 % clay	(Drillia et al. 2005)
Log K_{oc}	3.64	pH 6.8, TOC 0.37 %, 43.28 % clay	(Drillia et al. 2005)
Log K_{oc}	4.69	pH 6.6, TOC 2.2 %, CEC 28 $cmol^{-1}$ kg	(Yamamoto et al. 2009)
Log K_{oc}	3.21	pH 6.3, 3.77 % TOC, 20 % clay	(Barron et al. 2009)
Half-life in soil	>40 d	pH 6.25, OC 1%, CEC 5.2 $cmol^{-1}$ kg, 8 % clay	(Carter et al. 2014b)
PEC	44 ng L^{-1}		(AstraZeneca 2012)

2.2.3 Naproxen

Naproxen (Figure 2.3) is a non-steroidal anti-inflammatory drug (NSAID) that is used extensively (Table 2.5) to treat pain and inflammation in rheumatic disease, other musculoskeletal disorders, dysmenorrhea, and acute gout (British National Formulary 2015). Data from 2011 (most recent study available) shows it is the most used NSAID in Canada and the second most used NSAID in the UK, Bangladesh and the Philippines (McGettigan et al. 2013). The DDD is 0.5 g via the oral and rectal administration routes (WHO 2015a). The majority of naproxen is excreted via urine as naproxen (< 1 %), 6-0-desmethyl naproxen (< 1 %), and their glucuronide or other conjugates (66 - 92 %) (including (S)-naproxen acyl glucuronide, (S)-6-Odesmethyl naproxen acyl glucuronide and (S)-6-Odesmethylnaproxen sulfate); < 5 % is excreted in the faeces (Sugawara et al. 1978; USA FDA 2011).

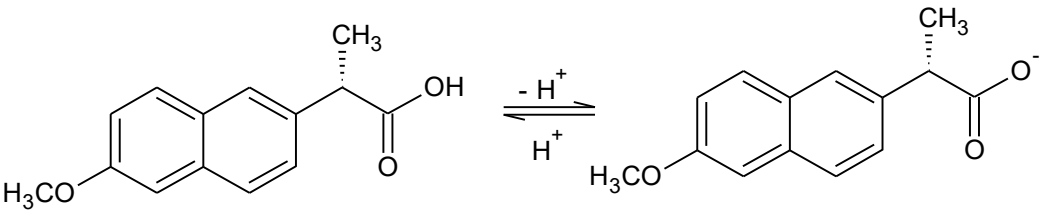


Figure 2.3 - Structures of naproxen and its anion.

Table 2.5 - Naproxen use as a percentage of total NSAID in selected countries for 2011 (McGettigan et al. 2013).

Country	Total NSAID usage in 2011 (DDDs Millions)	Naproxen use (% of total NSAID)	Naproxen use in 2011 (DDDs Millions)
Australia	297.4	13.3	39.6
Bangladesh	543.7	16.7	90.8
Canada*	12.9	28.2	3.6
China	346.1	1.5	5.2
China (Hong Kong)	63.9	10.0	6.4
England*	15.6	26.8	4.2
Indonesia	1173.1	0.0	0.0
Malaysia	111.5	4.2	4.7
New Zealand	63.5	20.5	13.0
Pakistan	1309.1	6.6	86.4
Philippines	199.3	21.2	42.3
Singapore	31.3	9.4	2.9
Taiwan	525.8	1.6	8.4
Thailand	447.2	3.3	14.8
Vietnam	103.8	0.1	0.1

*Use is expressed as sales of defined daily doses (DDDs, in millions) for all countries except England and Canada where it is expressed as millions of prescriptions dispensed in the community (McGettigan et al. 2013).

This API was chosen as it is negatively charged at environmental pH and is likely to be mobile in soils due to its low log K_{oc} (1.98 - 2.72) (Table 2.6 and Table 2.7). The log K_{oc} is under the trigger value of 4 so it does not require a terrestrial risk assessment, meaning that this API is less well studied than propranolol, for example (EMEA 2006) (Table 2.7). As naproxen is widely used globally (Table 2.5) it is likely to be present in many different WWTPs and potentially in the wider environment.

Table 2.6 - Properties of naproxen

Formula	$C_{14}H_{14}O_3$	
CAS	22204-53-1	
Purchase information	> 98.5 % purity, Sigma Aldrich	
Monoisotopic mass	230.094294 Da	
$[M+H]^+$	231.101571 Da	
[M-H]-	229.087018 Da	
Melting point	152-156 °C (experimental)	(ChemSpider 2015a)
Water solubility	< 3 g L^{-1} (experimental)	(ChemSpider 2015a)
pKa	4.15	(DrugBank 4.3 2013a)
$LogK_{ow}$	3.18 (experimental)	(U.S. National Library of Medicine)

Table 2.7 - Environmental parameters of naproxen

Property		Comments	Reference
WWTP removal	63 %	Bangkok; 2 stage activated sludge	(Tewari et al. 2013)
WWTP removal	59 %	Japan; activated sludge	(Nakada et al. 2006)
WWTP removal	87 %	Korea; activated sludge and final clarifier	(Sui et al. 2010)
WWTP removal	93 %	Sweden, activated sludge	(Bendz et al. 2005)
K_d soil	11	pH 6.3, 3.77 % TOC, 20 % clay	(Barron et al. 2009)
K_d soil	1.24	pH 7.54, organic matter 0.58 %, TOC 0.44 %, 3.6 % clay	(Xu et al. 2009b)
K_d soil	16.49	pH 7.14, organic matter 5.45 %, TOC 3.16 %, 18.1 % clay	(Xu et al. 2009b)
K_d soil	2.39	pH 8.01, TOC 25 mg g^{-1}, clay 45 %	(Durán-Álvarez et al. 2012)
Log K_{oc}	2.48	pH 6.3, 3.77 % TOC, 20 % clay	(Barron et al. 2009)
Log K_{oc}	2.45	pH 7.54, organic matter 0.58 %, TOC 0.44 %, 3.6 % clay	(Xu et al. 2009b)
Log K_{oc}	2.72	pH 7.14, organic matter 5.45 %, TOC 3.16 %, 18.1 % clay	(Xu et al. 2009b)
Log K_{oc}	1.98	pH 8.01, TOC 25 mg g^{-1}, clay 45 %	(Durán-Álvarez et al. 2012)
Half-life in soil	7.6 d	0-20 cm, loamy sand	(Chen et al. 2013)
Half-life in soil	5.9 d	0-20 cm, sandy loam	(Chen et al. 2013)
Half-life in soil	17.4 d	pH 9.23, 0.16 % OC, CEC 8.2 $cmol^{-1}kg$, 4 % clay	(Lin et al. 2011)
Half-life in soil	69.3 d	pH 8.73, 0.33 % OC, CEC 22.2 $cmol^{-1}kg$, 25 % clay	(Lin et al. 2011)
PEC	440 ng L^{-1}		(Carlsson et al. 2006)

2.2.4 Nevirapine

Nevirapine (Figure 2.4) is an antiretroviral therapy drug used globally in the treatment of HIV/AIDS. Global usage data is not available for this API as it is currently on patent, making this information commercially sensitive; the WHO DDD is 0.4 g (WHO 2016). Less than 3% of the total dose is excreted as the parent compound in urine (DrugBank 4.3 2017). Despite its low excretion rate, nevirapine has been measured in wastewater, surface and ground waters globally (Table 2.9), suggesting that it is a persistent API presenting a potential global environmental risk (Prasse et al. 2010; Wood et al. 2015; Bradley et al. 2016; Ngumba et al. 2016). Nevirapine differs from the other three APIs in that it is amphoteric; it only has a weakly acidic amide hydrogen but three weakly basic nitrogen atoms available for protonation. No data on the concentration or fate of nevirapine in soils has been reported to date. This API was chosen because it has a wide global use and has not been studied in soils.

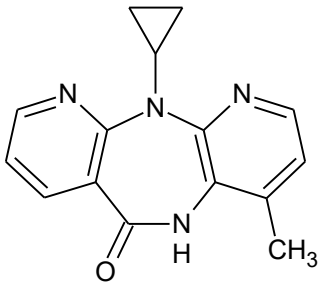


Figure 2.4 - Structure of nevirapine

Table 2.8 - Properties of nevirapine

Formula	$C_{15}H_{14}N_4O$	
CAS Number	129618-40-2	
Purchase information	98%, Acros Organics	
Monoisotopic mass	266.116761 Da	
$[M+H]^+$	267.124038 Da	
$[M-H]^-$	265.109485 Da	
Melting point	239-249 °C	(ChemSpider 2015b)
Water solubility	0.7046 mg L^{-1} (predicted)	(Madikizela et al. 2017)
pK_a	2.8	(Kasim et al. 2004)
$LogK_{ow}$	2.5	(Bagnis et al. 2018)

Table 2.9 - Environmental occurrence of nevirapine

Property		Comments	Ref
WWTP removal	11 – 49 %	Kenya, water stabilisation ponds or trickling filter	(K'Oreje et al. 2016)
WWTP removal	None	Germany, primary clarifier followed by biological treatment and chemical phosphorous removal	(Prasse et al. 2010)
River concentration	< 6 µg L^{-1}	Kenya, 3 rivers	(K'Oreje et al. 2016)
River concentration	360 ng L^{-1}	Average of 29 rivers across South Africa	(Wood et al. 2015)
River concentration	8 ng L^{-1}	Several wadeable streams in USA	(Bradley et al. 2016)
Groundwater concentration	1.2-1.6 µg L^{-1}	Shallow wells within informal settlements, Kenya	(K'Oreje et al. 2016)

2.3 Soil selection

Two soils were chosen according to OECD 106 (OECD 2000). These soils were purchased from Lufa Speyer (Germany) and had at least a 5 year history of no pesticide, biocidal fertiliser, or organic manure application, resulting in a soil that should be as 'clean' from contaminants as possible. This 'clean soil' meant that the analysis of soil solutions should be as free from potentially conflicting compounds and that all potential sorption sites were available for the APIs. They were provided air dried and sieved to < 2 mm. Sampling by Lufa Speyer was completed according to ISO 10381-6 (1993) and Good Laboratory Practices were followed. Table 2.10 shows the properties of the two soils, how they match with OECD 106 guidelines and their locations in lower and lower middle income countries (LLMIC) with similar soil properties. Both soils matched the requirements specified by the OECD other than the clay content of the loam soil, which was slightly higher than specified by the OECD (0.8 % over the maximum). The OECD 106 soil selection provides guidance and researcher judgement is needed to identify the suitability of the soils. It was decided to focus on two soils in these experiments due to experimental and analytical time constraints. Whilst this limits the depth of the conclusions that can be drawn from Chapter 4, sorption of APIs to soils, it allowed for the analysis of sorption of four APIs into soils from synthetic wastewater (Chapter 5).

Table 2.10 - Selected soils properties and comparison with OECD 106 (mean values of different batch analyses ± S.D. measured by LUFA Speyer) (OECD 2000; Lufa Speyer 2015)

	Sandy loam	Loam	OECD loamy sand	OECD silt loam
pH (0.01 M $CaCl_2$)	5.7 ± 0.6	7.3 ± 0.1	< 4.0 - 6.0	5.5 - 7.0
TOC (%)	0.7 ± 0.0	2.0 ± 0.2	< 0.5 - 1.5	1.5 - 3.0
Clay content (%)	6.5 ± 1.6	25.8 ±1.8	< 10 - 15	15 - 25
Cation exchange capacity ($cmol^{-1}$ kg)	7.5 ± 0.9	33.0 ± 4.5		
LLMIC examples with similar soil properties	SE China (Guangxi and Yunnan provinces). NW India (Odisha and West Bengal). NE Democratic Republic of Congo	Malaysia El Salvador		

2.4 Apparatus preparation and purchase information

All reusable plastic and glassware was thoroughly cleaned to reduce the input of other impurities into experiments. High purity water (HPW) was sourced from a Millipore system (18.2 MΩ.cm resistivity). Plastic and glassware was soaked in 2 % v/v Decon® for at least 24 hours, then rinsed with HPW, placed in 10 % v/v hydrochloric acid for at least 24 hours before rinsing with HPW and dried under a laminar flow hood. All acid-washed apparatus was stored in plastic bags. Glass fibre filter papers (0.7 µm, Fisherbrand, UK) were wrapped in aluminium foil and ashed in a furnace at 450 °C for 6 hours to remove any organic residues present before use.

Sterile centrifuge tubes (50 mL) were purchased from Fisher Scientific (UK); these were made of polypropylene plastic to minimise loss of APIs on the tube walls (section 2.5.2). Clear glass autosampler vials with silicon septa were purchased from Fisher Scientific. Sterile syringes were purchased from Fisher Scientific; they were

composed of polypropylene and polyethylene without latex, rubber silicone, styrene or DEHP. Filter holders were polypropylene. The recovery of APIs from apparatus was tested and data was corrected for any losses quantified.

2.5 Apparatus

2.5.1 Filter selection

Filters were required that could remove suspended solids from soil solutions for analysis, but not APIs. The pore size required was 0.7 µm or below for analysis by HPLC-HRAM-MS or fluorescence spectrometry. A comparison was made between API calibrations in HPW before and after filtration and recoveries were calculated. Stock solutions at three concentrations were made up separately for all APIs. An aliquot of this was then filtered and both samples were analysed in tandem. Three filter types were tested for propranolol, naproxen and ofloxacin; glass fibre (0.7 µm), polycarbonate (0.2 µm) and cellulose nitrate (0.2 µm). Nevirapine was added later to the experiment so only glass fibre filters were assessed and only two replicates at each concentration were analysed due to time limitations on the instrument. This meant that relative standard deviation could not be calculated (Figure 2.5). Glass fibre filters were chosen as they had the highest recovery for all three APIs (Figure 2.5).

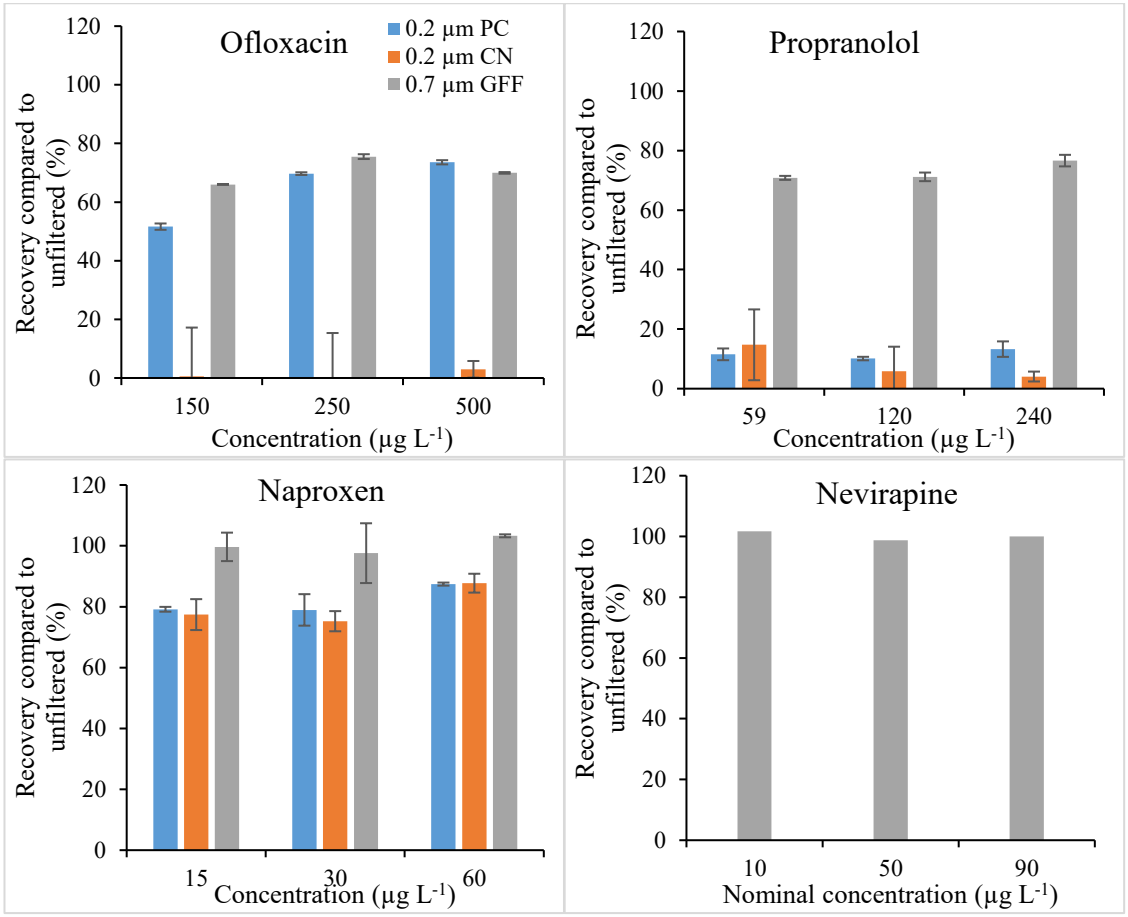


Figure 2.5 - Recovery of APIs from filters (PC – polycarbonate, CN – cellulose nitrate, GFF – glass fibre filter) ($\bar{x}$ ± RSD of filtered solution n = 3 apart from nevirapine where n = 2 so no RSD calculated). Legend is shown in ofloxacin graph and applies to all graphs in this figure.

2.5.2 Centrifuge tubes

To reduce API loss and reduce contamination, all sorption experiments were carried out in apparatus that could be centrifuged. Polypropylene centrifuge tubes were chosen as they could hold the correct amount of solution and soil with enough air space left for adequate mixing on the shaker, when placed horizontally.

To assess sorption of the APIs to the container walls a preliminary sorption experiment was undertaken without any soil in the tube. A 30 mL volume of 10 mM $CaCl_2$ (Fisher Scientific, UK) solution (in triplicate) was shaken overnight then spiked with API solution (Table 2.11), then shaken for a further 4 hours at room temperature.

Table 2.11 - Spikes and recoveries of APIs in 50 mL polypropylene centrifuge tubes ($\bar{x}$ ± S.D. n = 3)

	Spike concentration (µg L^{-1})	Recovery (%)
Ofloxacin	500	98.6 ± 4.2
Propranolol	240	101.4 ± 0.8
Naproxen	58	91.7 ± 17.1
Nevirapine	50	115 ± 3.7

The recoveries of the APIs from the centrifuge tubes were acceptable and errors were within experimental variations allowable by SANCO guidelines (error ≤20 %) (European Commission 2000; 2010). As a result all sorption experiments were performed in polypropylene centrifuge tubes.

2.6 Statistical analyses of data

The Shapiro Wilk test was used to test the goodness of fit to a normal distribution for all data. This test is good to use on small sample sizes and all tests were undertaken at a significance level of $p \leq 0.05$ (Marques de Sá 2003). All samples were found to fit a normal distribution allowing the parametric statistical tests of analysis of variance (ANOVA) and T-tests to be used throughout.

The Shapiro Wilk test is calculated following equation 2.1. This test provides a *W* value; where small values indicate the sample is not normally distributed, the null hypothesis can be rejected, and a high *W* value suggests the sample is normally distributed when compared to a table of critical values.

2.1

$$W = \frac{\left(\sum_{i=1}^{n} a_i x_{(i)}\right)^2}{\sum_{i=1}^{n}(x_i - \bar{x})^2}$$

Where *W* = Shapiro-Wilk value, n = number samples, a_i = constants (generated from look-up-tables), x_i = ordered random sample values

2.7 Fluorescence spectrophotometry

Fluorescence spectrophotometry was initially used for analysis of samples containing propranolol, naproxen and ofloxacin due to its ease of access and low cost during the sorption method development stage. Basic analysis involved placing filtered solutions into a quartz glass cuvette (Hellma fluorescence cuvette 200-2500 nm spectral range, purchased from Sigma Aldrich) and analysed in 3D scan mode using a Hitachi F-4500 fluorescence spectrophotometer (Table 2.12). Initially scans at a large excitation/emission range were undertaken in HPW spiked with a single API and compared with blank HPW to measure its response has under fluorescence.

Table 2.12 - Hitachi F-4500 fluorescence spectrophotometer parameters

Excitation sampling interval	10 nm
Emission sampling interval	10 nm
Scan speed	2400 nm/min
Excitation slit	5.0 nm
Emission slit	5.0 nm
PMT Voltage	950 V
Response	0.004 s
Corrected spectra	Off
Shutter control	Off

Propranolol, naproxen and ofloxacin fluoresced over a range of excitation and emission wavelengths (Figure 2.6). A range of three API concentrations was used to calculate the LOD for the three APIs using the concentrations in Table 2.13. These solutions were made in HPW and both soil filtrates, then analysed in triplicate on the day of spiking. LODs for these APIs was calculated in HPW and spiked filtered soil filtrates (Table 2.14) using Equation 2.2 after measurements were corrected for blank matrix effects (Figure 2.6).

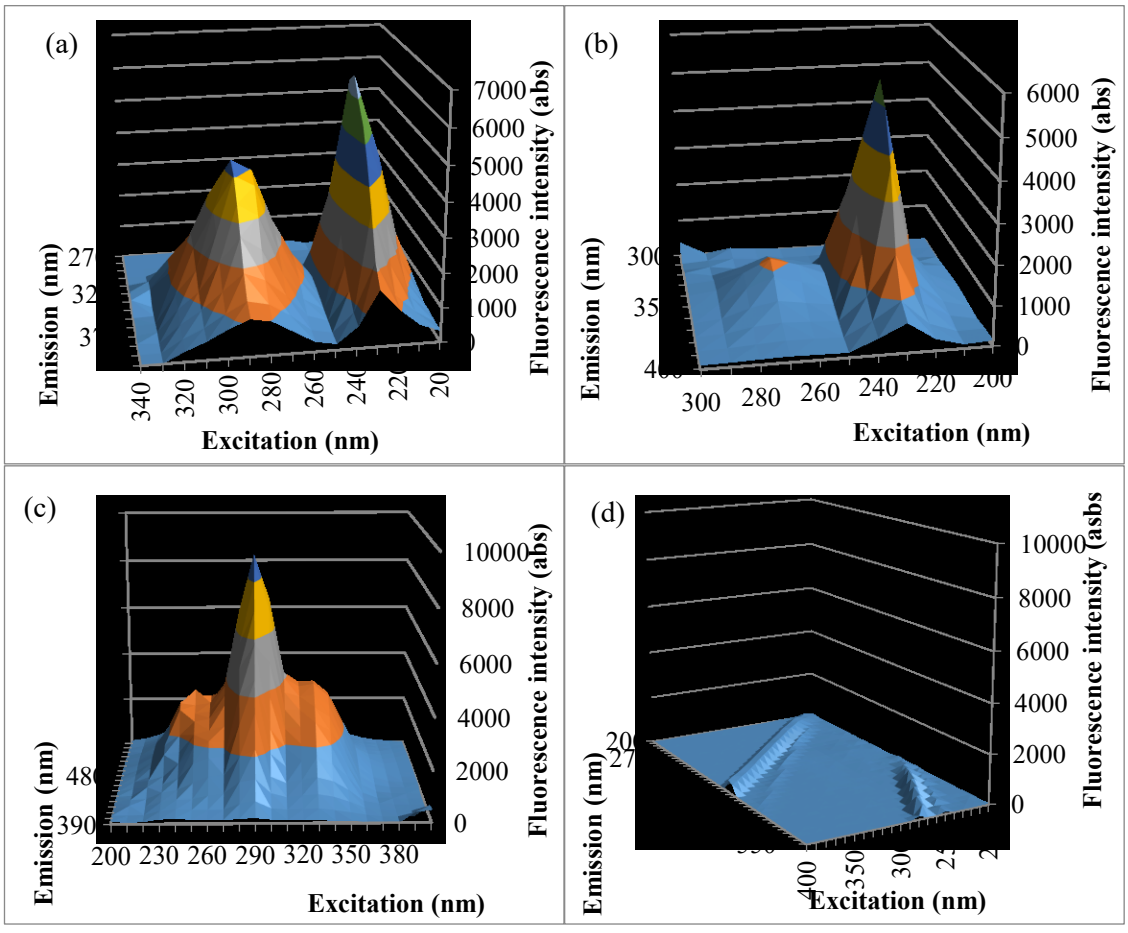
Table 2.13 - Concentrations of APIs used to produce calibration lines to calculate LOD for fluorescence spectrophotometry

	Low (µg L^{-1})	Middle (µg L^{-1})	High (µg L^{-1})
Ofloxacin	150	250	500
Propranolol	59	120	240
Naproxen	15	30	60

2.2

$$LOD = \frac{(3 \times blank\ standard\ deviation)}{slope}$$

All calibration graphs showed a linear response ($R^2 \geq 0.98$) for the concentration range and matrices used.



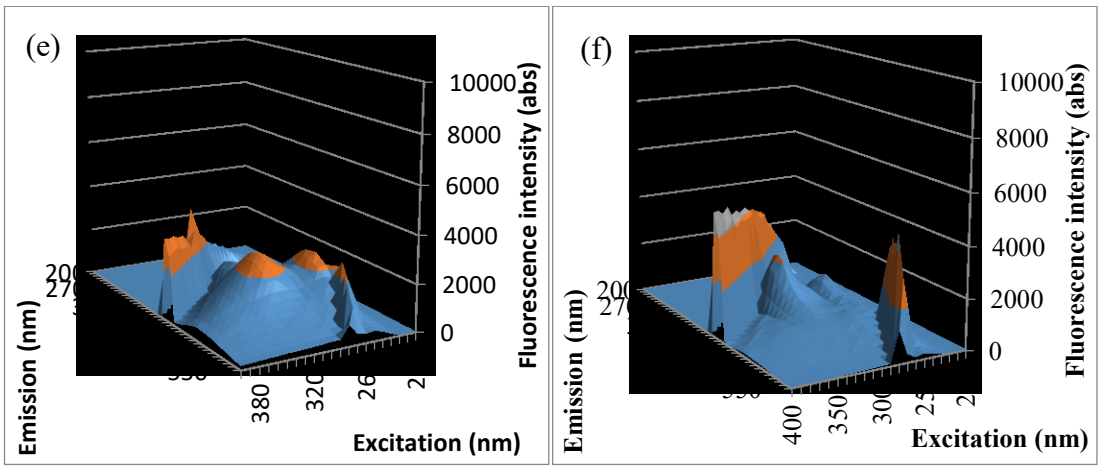


Figure 2.6 - Typical fluorescence responses of APIs in HPW (a) Propranolol (240 µg L^{-1}) (b) Naproxen (60 µg L^{-1}) (c) Ofloxacin (500 µg L^{-1}) (d) HPW (e) Loam soil filtrate (f) Sandy loam soil filtrate

Table 2.14 - Limits of detection (LODs) for analytes in a range of matrices using 3D fluorescence spectrophotometry

	Propranolol		Naproxen		Ofloxacin
Excitation/Emission (nm)	230/340	290/340	230/350	270/350	290/460
LOD – HPW (µg L^{-1})	3.1	6.7	1.4	5.9	0.4
LOD – Loam soil solution (µg L^{-1})	8.3	37.3	1.7	2.5	2.8
LOD – Sandy loam soil solution (µg L^{-1})	0.9	1.5	0.6	1.9	2.5

There were a number of limitations associated with this method for determination of APIs in environmental samples:

- As it was a static method, only one type of datum was recovered; this was a signal intensity that was then compared with a calibration curve to quantify concentrations in solution (external calibration). This contrasts with high pressure liquid chromatography-mass spectrometry (HPLC-MS) where additional data on compound mass and degradation products can be obtained to enable accurate identification and quantification of the API, and to assess the stability of an API in soils over the course of the sorption experiment.

- Only one API could be determined at a time, because the excitation and emission wavelengths were close together, especially for propranolol and naproxen.
- The LOD for each API was higher than achievable using HPLC-MS.
- The matrix could affect the API signal where there was overlap in excitation/emission peaks, resulting in a high blank value.
- Quenching of the API signal by the matrix was observed and needed to be accounted for by analysing matrix-matched calibration standards.

However, fluorescence spectrometry did offer some advantages over HPLC -MS:

- It was easy to set up and use, providing a rapid sample turnaround time compared to HPLC- MS.
- The LODs were low enough to allow measurement of the APIs in the μg L^{-1} range.
- No additional solvents were required.
- Furthermore, the instrumentation is available in many laboratories and is significantly cheaper than HPLC-MS.

2.8 Liquid chromatography – mass spectrometry

2.8.1 Background

The development of methods for the analysis of APIs in soil matrices has been growing slowly over the past decade, much more slowly than the comparative methods available for aqueous matrices (Białk-Bielińska et al. 2016). This is due to the need to

initially extract the residues sorbed to soil particles before quantifying the concentrations present (Wilga et al. 2008). As the method used in the current research does not involve the extraction of APIs from the soils this is not discussed here. High-pressure liquid chromatography-mass spectrometry (HPLC-MS) has been used as a reliable and accurate method of detecting and quantifying APIs in soil extracts for several years (Díaz-Cruz et al. 2007; Wilga et al. 2008).

A high-pressure liquid chromatography-high resolution accurate mass-mass spectrometer (HPLC-HRAM-MS) was used to detect and quantify the APIs. HPLC-HRAM-MS analysis was conducted using an Ultimate U3000 UHPLC liquid chromatography system and a Q Exactive Focus Orbitrap mass spectrometer (both Thermo Scientific) (Figure 2.7). Gaseous phase ions were generated from solution by electrospray ionisation before detection by mass spectrometry, allowing for the sensitive analysis of both ionised species and neutral compounds (Ho et al. 2003).

The instrument was chosen due to its high resolution accurate mass detection and high sensitivity, allowing APIs to be positively identified at low concentration in different matrices. High resolution accurate mass detection reduced sample preparation, allowing for lower waste solvent volumes and shorter analysis times as solid phase extraction or pre-concentration were not required at test concentrations.

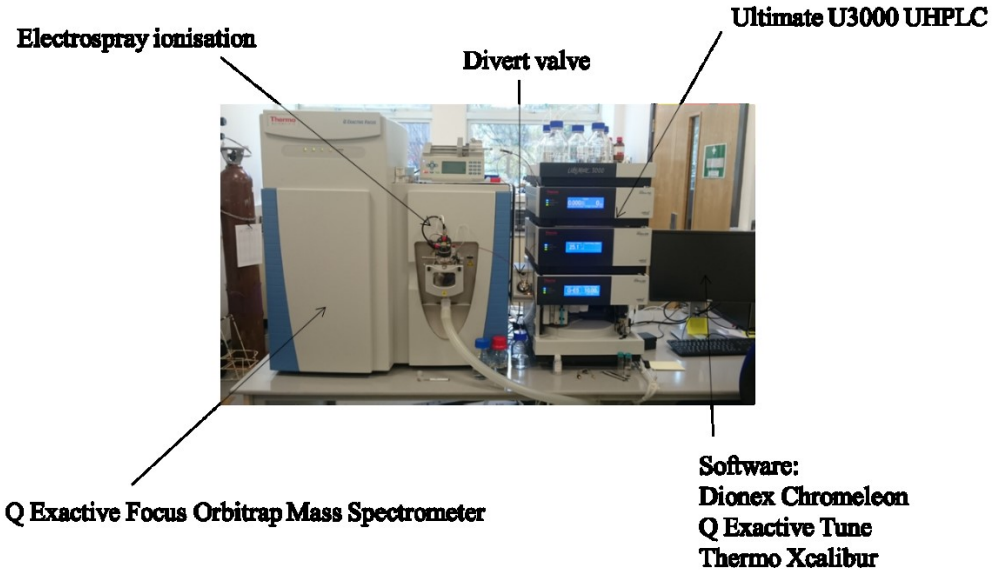


Figure 2.7 - HPLC-HRAM-MS instrument used in this study, with individual components identified

2.8.2 HPLC-MS method development

Method development was conducted over several months to create and optimise a single analytical method for the four APIs used in the experiments. This included assessing ionisation mode (positive or negative), eluents, columns, needle wash and scanning method. The main problems encountered were carry-over of ofloxacin and propranolol and changing sensitivities of the instrument over time.

Three C18 HPLC columns were used throughout the course of this research for the liquid chromatography, initially starting with a Kinetex column before changing to two different Waters columns to reduce back pressure and improve peak shape. The columns used were a Kinetex 2.6 µm EVO C18 100 Å (2.1 x 100 mm), a Waters XBridge BEH C18 2.5 µm (2.1 x 50 mm) Column XP and a Waters XSelect CSH C18 2.5 µm (2.1 x 50 mm); all columns had a pre-column filter fitted (HiChrom 0.5 µm). As a result of the instrument's sensitivity an injection volume of 5 µL was chosen to reduce carryover,

while the injector needle wash, performed before and after each injection, was 1:1 (v/v) HPW : methanol.

Different eluent additives were tested, namely 0.1 % (v/v) ammonia or formic acid in HPW. Ammonia addition optimised sensitivity to the APIs but increased carry over (mainly ofloxacin and propranolol); as a result formic acid was elected as the additive. Methanol was chosen as the organic solvent for the eluents.

Gradient elution was performed using water (0.1 % v/v formic acid) and methanol (C and D, respectively) as mobile phase solvents. The gradient program ran from 95 : 5 to 0 : 100 C : D over 6 minutes, then held for 2 minutes before returning to the starting ratio for 2 minutes (Figure 2.8).

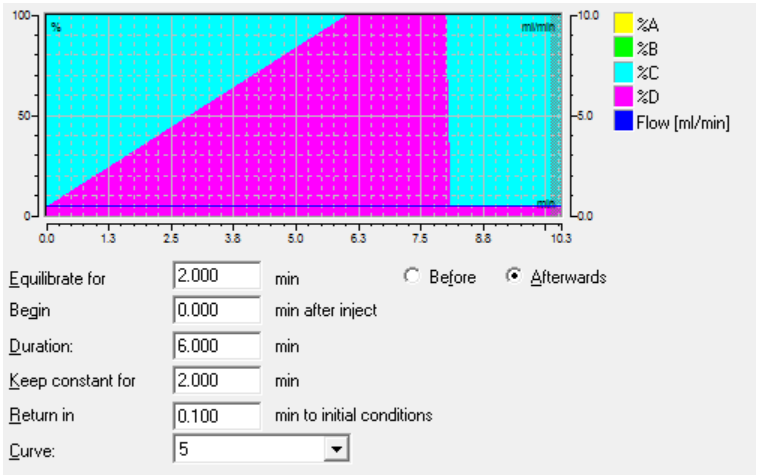


Figure 2.8 - HPLC gradient conditions (C = water amended with 0.1 % v/v formic acid; D = methanol)

The retention times (Figure 2.9) show that the APIs were completely resolved from each other in this gradient elution programme, and thus could be spiked into soil solutions as a mixture, reducing both sample processing and analysis time.

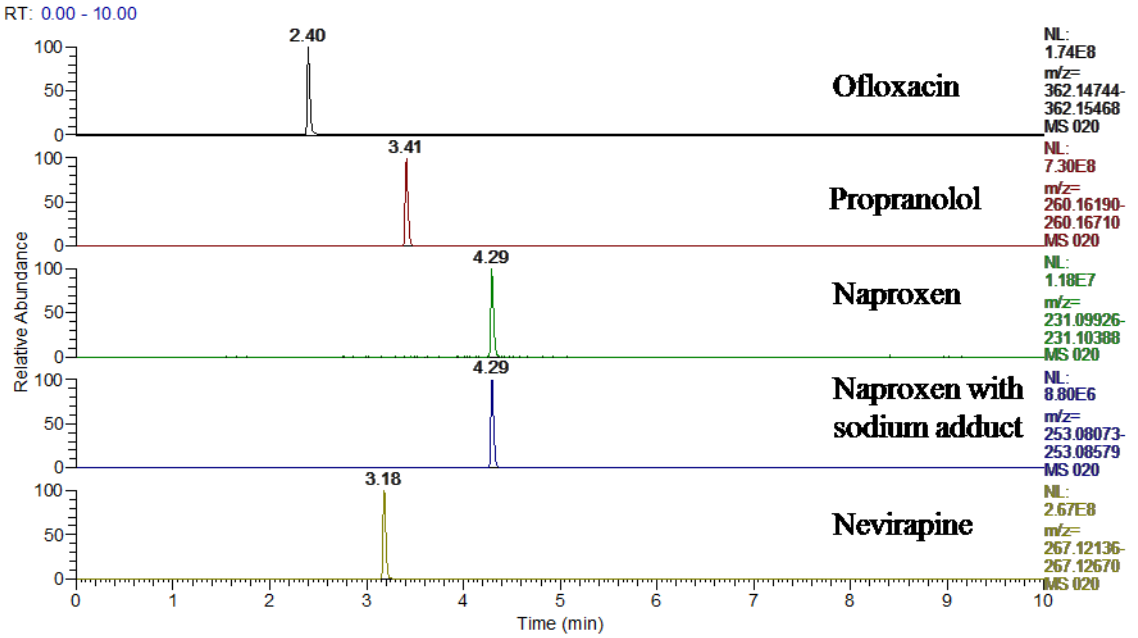


Figure 2.9 – Example chromatogram of 4 APIs spiked at 100 µ gL^{-1} in HPW (chromatogram produced 07/08/2017 using Waters XBridge BEH C18 2.5 µm 2.1x50 mm Column XP)

The eluent stream was diverted to waste from 0.1 – 1.5 minutes of the analytical run before entering the mass spectrometer in order to reduce contamination of the ionization source with salts from the sample matrix.

All mass spectrometric analyses were performed in the positive ion mode. Parallel reaction monitoring (collision energy 30 eV) was used initially to target the specific ions and enable confident API confirmations away from the background. Once it was apparent that the soil matrix did not interfere with the mass spectrum analysis, full scan mode (*m*/*z* 100-1000) was used for all analyses.

Prior to analysis, the mass spectrometer was mass evaluated and calibrated using an external injection of a positive ion calibration solution (n-butylamine, caffeine, MRFA, and Ultramark 1621, Pierce LTQ Velos ESI, Thermo Fisher Scientific, UK). Methanol (HiPerSolv Chromanorm, VWR, UK) and HPW containing 0.1 % (v/v) formic acid

(Optima Thermo Fisher Scientific, UK) were added and purged through the system. A new needle wash of 1:1 (v/v) HPW : methanol was also added.

2.8.3 Data analysis

Raw data were analysed using Xcalibur software (Thermo, UK). All samples were analysed at least in duplicate. Peaks were detected and smoothed using ICIS Peak Integration (Figure 2.10) and the processing method 2016_03_18FS100-1000XBridge. The APIs were identified using a 5 decimal place *m/z* and the expected retention time. Sodium is a common contaminant in HPLC-MS and usually enters the sample through lab glassware and eluents used in HPLC. As naproxen also forms a sodiated adduct, the peak area of the parent ion and the sodiated adduct were combined to account for differing levels of sodium in the eluents.

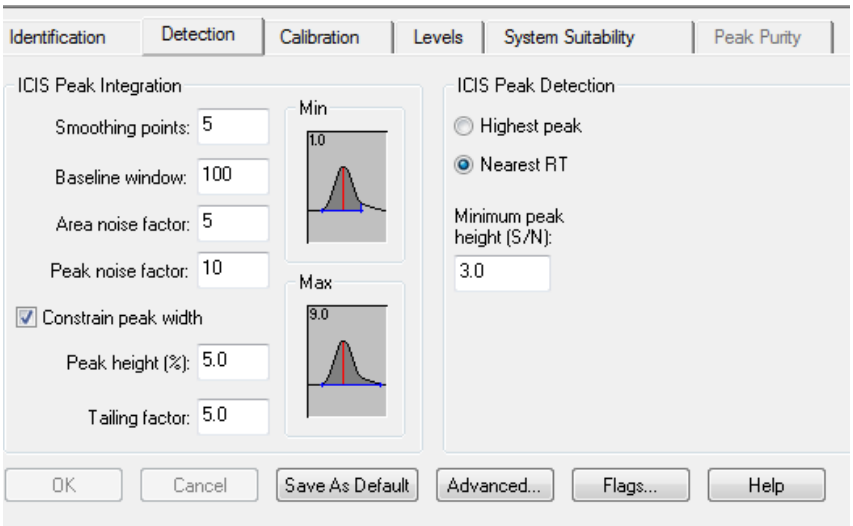


Figure 2.10 - Peak detection and processing method from Xcalibur software (Thermo)

Calibration standards included at least six separate concentrations and spanned at least two orders of magnitude (Table 2.15). Because of the wide range in the concentrations of the standards and with this loss of homoscedasticity, calibration lines

were weighted, using the Excalibur software, to adjust the best fit line by a factor related to an inverse function of the concentration, namely 1/x (Kiser et al. 2004). This served to reduce the relative error (variance) of each standard calibration measurement and made the relationship more relevant (reduced bias) at the lower end of the concentration range (Gu et al. 2014). All API calibrations were linear (apart from propranolol in solutions in synthetic wastewater (SWW)), had an R^2 greater than 0.99 (apart from ofloxacin in SWW) (Table 2.16) and a percentage difference between the calculated and nominal concentrations of below 15 %.

Table 2.15 - Calibration concentrations for each API (µg L^{-1})

Cal label	Ofloxacin	Propranolol	Naproxen	Nevirapine
C1	0.1	0.8	0.3	0.4
C2	0.625	5	1.875	2.5
C3	1.25	10	3.75	5
C4	5	40	15	20
C5	10	80	30	40
C6	20	160	60	80
C7	25	200	75	100

Table 2.16 – Calibration data in all matrices

API	Matrix	Equation	R^2
Ofloxacin	HPW	$y = 2.48x10^6 \chi + 802007$	0.9945
	Loam	$y = 2.21\ x10^6 \chi + 105035$	0.9925
	Sandy loam	$y = 2.75\ x10^6 \chi + 18175$	0.9997
	SWW	$y = 0.93\ x10^6 \chi + 930210$	0.9689
	SWW + loam	$y = 0.76\ x10^6 \chi + 25681$	0.9917
Propranolol	HPW	$y = 7.87x10^6 \chi + 3.66x10^6$	0.9925
	Loam	$y = 7.51x10^6 \chi + 4.87x10^6$	0.9950
	Sandy loam	$y = 7.51x10^6 \chi + 2.08x10^7$	0.9934
	SWW	$y = 6.20x10^6 \chi - 5552 \chi^2 + 1.27x10^6$	0.9994
	SWW + loam	$y = 8.11x10^6 \chi - 7194 \chi^2 + 2.37x10^6$	0.9981
	SWW + sandy loam	$y = 6.34x10^6 \chi - 6409\ \chi^2 + 0.8\ x10^6$	0.9995
Naproxen	HPW	$y = 0.29x10^6 \chi - 464824$	0.9981
	Loam	$y = 0.27x10^6 \chi - 438441$	0.9986
	Sandy loam	$y = 0.25x10^6 \chi - 453599$	0.9984
	SWW	$y = 0.42x10^6 \chi - 74745$	0.9994
	SWW + loam	$y = 0.46x10^6 \chi - 51935$	0.9992
	SWW + sandy loam	$y = 0.41x10^6 \chi - 74212$	0.9997
Nevirapine	HPW	$y = 5.13x10^6 \chi - 199410$	0.9993
	Loam	$y = 0.26x10^6 \chi - 438441$	0.9986
	Sandy loam	$y = 5.24x10^6 \chi + 445596$	0.9986
	SWW	$y = 4.45x10^6 \chi + 69675$	0.9995
	SWW + loam	$y = 6.82x10^6 \chi + 1.93x10^6$	0.9955
	SWW + sandy loam	$y = 4.84x10^6 \chi + 345717$	0.9986

2.8.4 Limit of detection and quantitation

Instrumental limits of detection (LOD) and quantitation (LOQ) were calculated using data collected in August and September 2017. At least 5 low calibration concentrations were used to ensure that the data presented was robust and analytically relevant. Both of these limits were calculated using ICH methods (Equations 2.3 and 2.4) (ICH Harmonized Tripartite Guideline 2005).

2.3

$$LOD = \frac{(3.3\,\sigma)}{S}$$

Where σ = standard deviation of the y-intercepts of the regression line and S = slope of the calibration curve.

2.4

$$LOQ = \frac{(10\,\sigma)}{S}$$

LODs and LOQs that were calculated for this instrumental method were adequate for the experiments that follow (Table 2.17 and Table 2.18).

Table 2.17 - Limits of detection for APIs in different matrices (µg L^{-1})

	HPW	Loam	Sandy loam	SWW	SWW Loam	SWW sandy loam
Ofloxacin	0.36	0.16	0.14	0.94	0.46	N/A
Propranolol	0.39	1.95	0.51	0.80	1.06	0.46
Naproxen	0.14	0.82	0.99	0.14	0.36	0.21
Nevirapine	0.15	0.28	0.25	0.07	0.72	0.09

Table 2.18 - Limits of quantitation of the APIs in different matrices (µg L^{-1})

	HPW	Loam	Sandy loam	SWW	SWW Loam	SWW sandy loam
Ofloxacin	1.08	0.49	0.42	2.83	1.40	N/A
Propranolol	1.18	5.90	1.53	2.41	3.21	1.40
Naproxen	0.44	2.49	3.00	0.44	1.08	0.63
Nevirapine	0.45	0.84	0.76	0.23	2.19	0.26

2.8.5 Precision of HPLC-HRAM-MS analytical method

Precision refers to the closeness of agreement between measured values on the same or similar samples under specified conditions (JCGM 2012). The repeatability precision of the analytical measurements was calculated to quantify random error over the length of an analytical run. To achieve this, the relative standard deviation (RSD) was calculated at three concentrations in HPW using at least 7 repeat measurement (Equations 2.5 and 2.6) (European Commission 2002). Due to problems with degradation or loss of ofloxacin to the glass autosampler vials, a batch of standards was made up in advance and frozen in 1 mL aliquots and defrosted alongside samples in extended periods of analysis longer than 48 hours.

2.5

$$s = \sqrt{\frac{\sum_{i=1}^{n}(x_i - x_{mean})^2}{n-1}}$$

2.6

$$RSD\ (\%) = \left(\frac{s}{x_{mean}}\right) \times 100$$

Where s = sample standard deviation, n = number of measurements made, x_i = each measurement result, x_{mean} = mean value of the measurement results, RSD = relative standard deviation

The APIs, except for ofloxacin, showed good precision according to the EU Directive 2002/657/EC as each RSD was below 20 % (Table 2.20(European Commission 2002). This Directive specifies common criteria for the interpretation of analytical results to ensure data are comparable between official laboratories and samples. Ofloxacin failed at the lower concentrations because the concentration used was below the instrumental limit of quantitation (< 2.61 μg L^{-1}) (Table 2.18). Ofloxacin had a higher RSD than the other APIs but this was related to the lower concentrations used to calculate it. Ofloxacin has a tendency to sorb to glass vials which is more pronounced at lower concentrations (Ciarlone et al. 1990). As the samples used for the precision experiment were in the glass vials of the autosampler for different periods of time, loss of ofloxacin to glass vials would have varied.

Table 2.19 - Concentration of APIs used to calculate precision

	Low (μg L^{-1})	Middle (μg L^{-1})	High (μg L^{-1})
Ofloxacin	1.25	10	25
Propranolol	10	80	200
Naproxen	7.5	60	150
Nevirapine	7.5	60	150

Table 2.20 – Relative standard deviation of HPLC-HRAM-MS method for APIs at low, middle and high concentrations

	Low (%)	Middle (%)	High (%)
Ofloxacin	94	23	17
Propranolol	1	3	6
Naproxen	4	4	4
Nevirapine	3	4	4

2.8.6 API and sample storage

The aim of this experiment was to test that the samples could be successfully stored at -20 °C without effecting the concentration of API in the solutions. This was necessary as the HPLC-HRAM-MS was regularly busy so adequate sample preservation was required until it was available for use.

Freshly made mixed stock solutions of the APIs were made in HPW and diluted into a calibration range (0.1, 1, 10, 50, 100 µg L^{-1}). These were analysed and compared against a calibration range that had been made from individual API stocks in HPW and stored for a minimum of 6 months at -20 °C.

Ofloxacin was effected by the freezing of the solutions at 1-100 µg L^{-1} (ANOVA, $p \leq 0.05$). The differences between the two calibrations for ofloxacin ranged from 15-34 % with no trend depending on concentration. The largest observed difference occurred at 10 µg L^{-1} and the lowest at 100 µg L^{-1} (Figure 2.11). All of the APIs other than ofloxacin showed no storage effect, apart from random concentrations (propranolol at 0.01 and 10 µg L^{-1} and nevirapine at 100 µg L^{-1}) (ANOVA, $p \leq 0.05$).

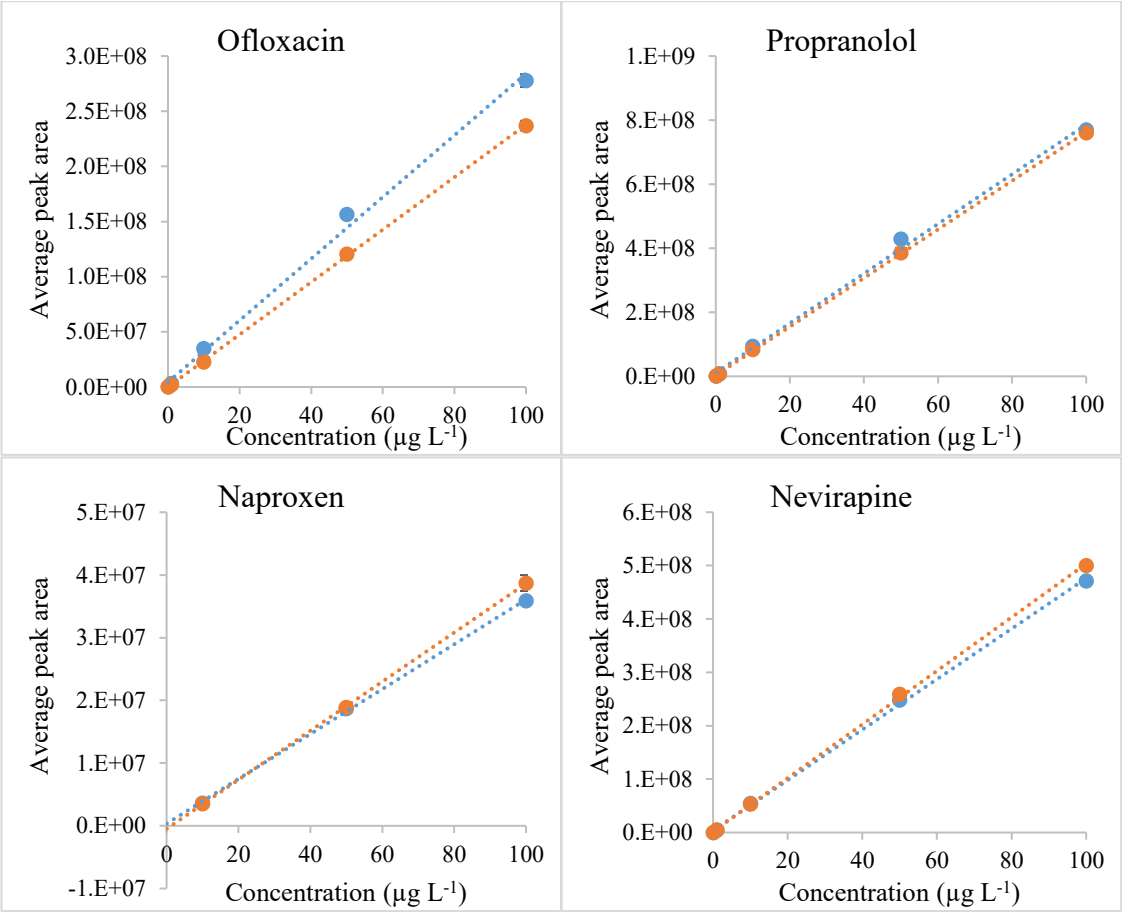


Figure 2.11 - Effects of storage on API calibrations. ● = freshly prepared, ● = frozen. Error bars were calculated but are not visible in most cases ($\bar{x}$ ± S.D. n = 3)

Based on these data, fresh API calibration solutions were used to test the sensitivity efficiency and precision of the instrument. If samples were stored at -20 °C, a set of standard solutions was prepared alongside samples and stored in the same manner for consistency.

2.8.7 Chromatographic resolution of APIs

The aim of this experiment was to check that there were no peak interferences between APIs so that a mixed spike of APIs could be added to soil solutions. Freshly prepared individual API stocks were made in HPW and diluted to a calibration range (0.1, 1, 10, 50, 100 µg L^{-1}) using HPW. These were analysed and compared with a freshly-

prepared mixed calibration range in HPW to assess the suitability of preparing and using a mixed API stock solution.

The results indicated that combining all four APIs into one spike did not affect the instrument response (Figure 2.12). Variation is accounted for by normal pipetting and weighing or analytical errors (section 2.8.5).

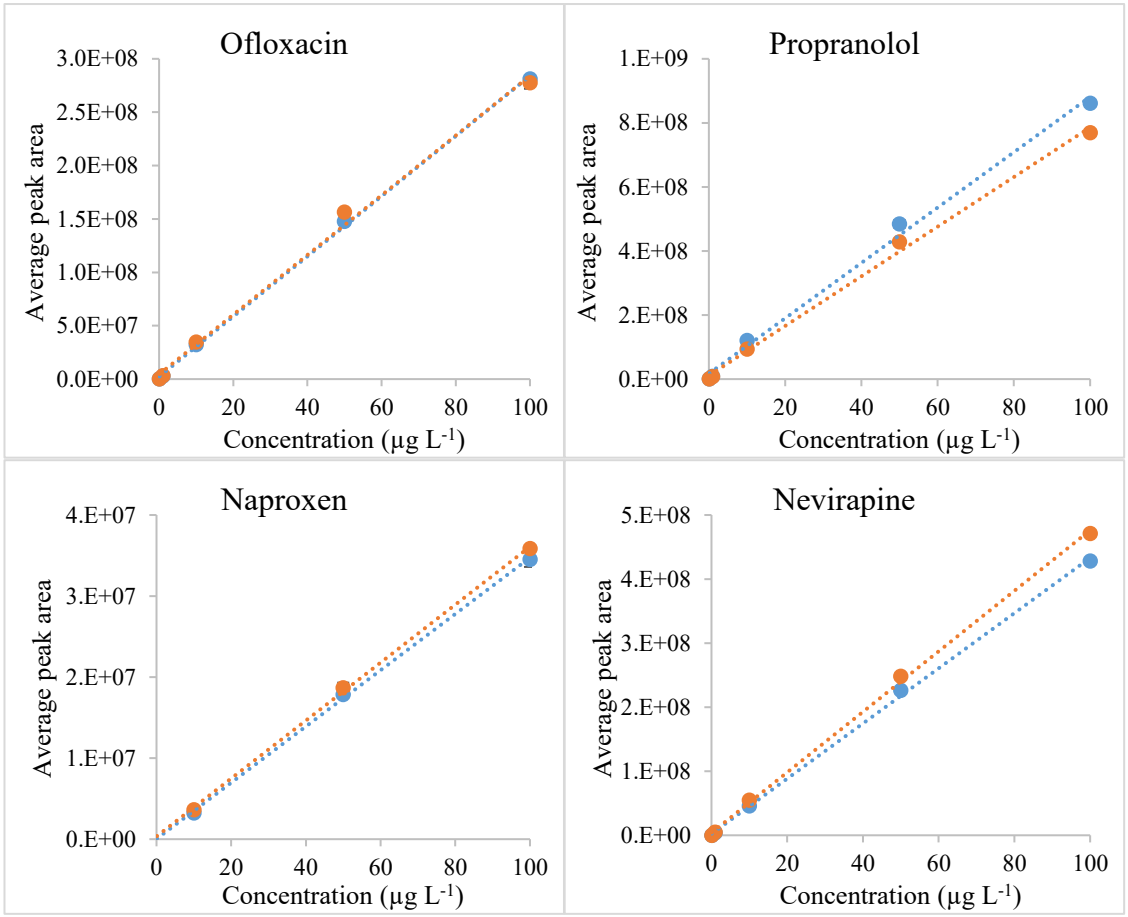


Figure 2.12 - API calibrations comparing mixed standards to individual standards. ● = individual standard, ● = mixed standard. Error bars were calculated but are not visible ($\bar{x}$ ± S.D. n = 3).

2.8.8 Matrix effects

The aim of this experiment was to analyse if the matrices of the soil solutions and synthetic wastewater (SWW) had an effect on the analysis of the APIs. This was tested by spiking filtered soil solutions in either 10 mM $CaCl_2$ or SWW matrix (the soil solutions were prepared by shaking overnight, centrifuging 4000 RPM 15 mins and filtering 0.7 μm GFF) at different concentrations and assessing the linearity and suppression of peak areas at each concentration compared to spiked high purity water. A HPW set of calibration solutions was prepared at the same time using the same API stocks for comparison.

Matrix effects were observed but these were not consistent across the four APIs. Where an effect was identified it was always quenching of the signal (Figure 2.13). Ofloxacin in sandy loam in both SWW and 10 mM $CaCl_2$ had the greatest matrix effect compared to HPW, with an 80 % decrease in the average peak area measured. These data indicated that calibrations should be performed in matrix matched solutions due to the signal quenching.

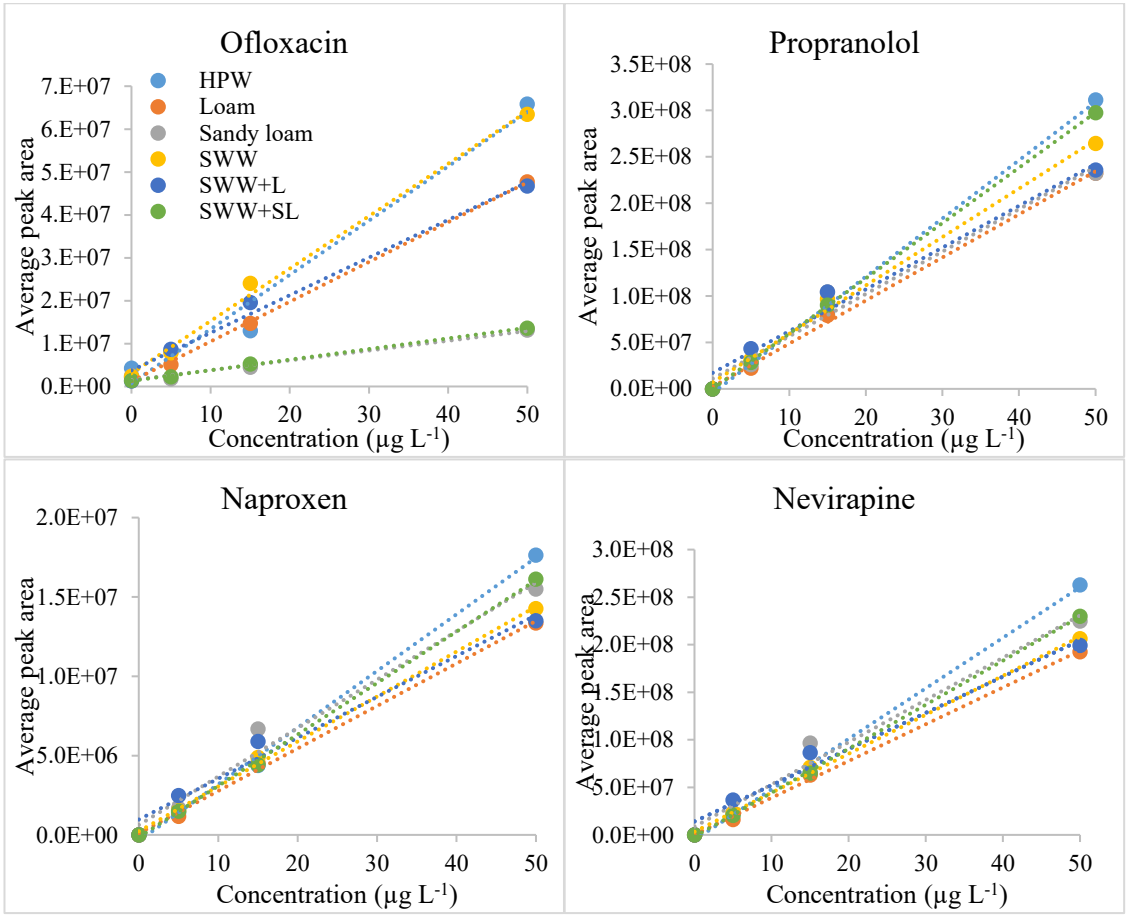


Figure 2.13 - Soil solution matrix effects. Legend is shown in ofloxacin graph and consistent throughout this set of graphs. Error bars are present but not visible ($\bar{x}$ ± S.D. n = 3)

2.8.9 120 hour soil solution matrix effect

The aim of this experiment was to check that the changes in the soil matrix that occur over the 120 hour sorption experiment do not affect the analytical API measurements. Calibrations were made in soil solutions that had been shaken overnight or for 120 h, commensurate with the longer sorption experiments described elsewhere. The soil solutions were centrifuged and filtered as described in section 2.8.8 and APIs then spiked at a range of concentrations.

The results, summarised in Figure 2.14, show that shaking time did not affect analyte recovery. The measured variation (e.g. ofloxacin in loam soil) was below 20 % and could potentially be attributed to pipetting errors or instrumental errors.

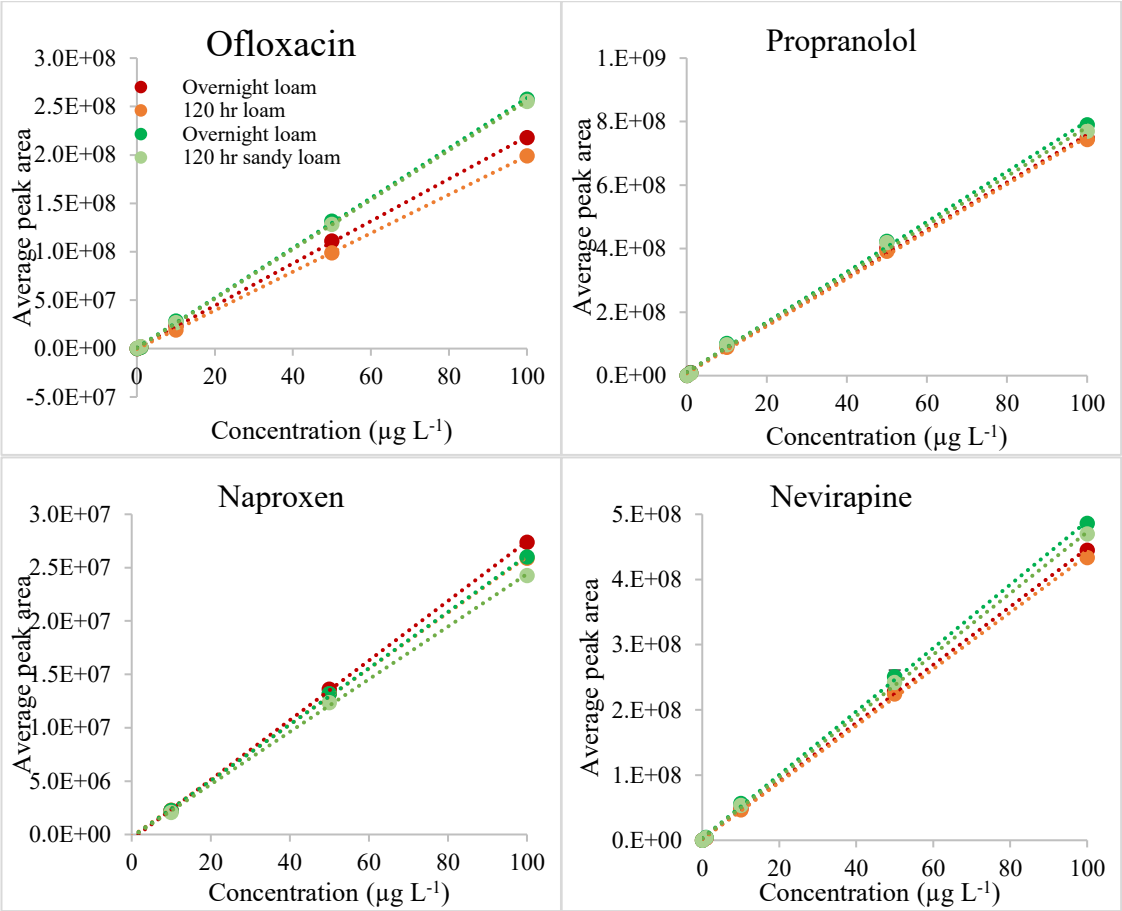


Figure 2.14 - 120 hour soil matrix effects. Legend is shown in ofloxacin graph and consistent throughout this set of graphs. Error bars are present but not visible ($\bar{x}$ ± S.D. n = 3)

Based on the measurements, and for ease of analysis, all experiments were calibrated in soil solutions representing the overnight matrix. Whilst there was some variation in specific APIs, the data was generally consistent across the time period and adding another calibration to the analysis with a 120 h matrix would have significantly increased instrument time.

2.9 Standard soil methodologies

To identify temporal changes in soil solutions, four soil characteristics were measured; namely pH, and overall soil charge, dissolved organic carbon and humic and fulvic acid components.

2.9.1 pH and soil charge

Soil pH was measured using a HANNA HI 9025 microcomputer pH meter fitted with a Camlab epoxy tough single junction combination pH electrode. This was calibrated using pH 4.01 and pH 7.00 buffers (Thermo Scientific). Experimental soil pH was measured in 10 mM $CaCl_2$ solutions (30 mL) containing 6 g soil, as this was the ratio used for sorption experiments. The use of a salt solution to measure pH in soils achieved standardisation of the method (Kah et al. 2007b)

The pH of 10 mM $CaCl_2$ solution can also provide information about the overall charge of a soil when compared to pH measured in water (Rowell 1994). 5 g of soil was shaken for 15 minutes with 25 mL reverse osmosis water or 10 mM $CaCl_2$ solution, this was done five times for each soil. The pH was measured after the probe had been in contact with the soil solution for 30 seconds. A soil with a net negative charge was expected to have a pH approximately 0.5 pH units higher in water than in the $CaCl_2$ solution, due to displacement of H^+ from the soil.

2.9.2 Dissolved organic carbon

Dissolved organic carbon (DOC) was measured using a Shimadzu TOC-V analyser, fitted with an ASI autoanalyser, following the method described by Badr et al (2003). Prior to analysis samples were filtered (0.7 µm ashed glass fibre filters) and acidified (10 µL 6 M HClper 10 mL sample). Acidified HPW was used if samples required dilution.

Filtered, acidified, soil solutions were loaded into the autosampler in combusted glass vials. Samples were sparged using bottled, high purity oxygen (ca. 8 min at 75 mL/min) to remove dissolved inorganic carbon (Badr et al. 2003). Samples were then injected onto a combustion column and the DOC oxidised to CO_2 at 650 °C in the presence of a Pt impregnated aluminium oxide catalyst. Combusted gases were dried

using an electronic dehumidifier and purified using a halogen scrubber (Badr et al. 2003). CO_2 was detected using a non-dispersive infrared detector (NDIRD). The signal from the NDIRD was recorded as voltage and peak measurement was used for quantification of DOC.

DOC standards were made using potassium hydrogen phthalate with a concentration range of 0 – 1690 μmol L^{-1} C. A straight line calibration graph was plotted and this was used to convert the peak area of samples into DOC concentration.

2.9.3 Humic and fulvic soil components

Humic and fulvic acid components of the soils were analysed using fluorescence spectrophotometry and compared with reported values (Chen et al. 2003). The scan type was 3D. A scan size of 200 - 600 nm excitation and 200 - 600 nm emission was used initially before being adjusted after peaks were identified in samples.

3 Assessing different soil sterilisation methods for use in OECD 106

3.1 Overview

Three soil sterilisation or microbial enzyme suppression methods were investigated to identify how successful they were at reducing soil enzyme activity and if there was any impact on the soil physical chemical structure. Gamma irradiation, autoclaving and the addition of 0.2 g L^{-1} sodium azide were studied as three standard methods used for sterilisation. The results from this experiment were discussed in the context of the OECD 106 batch sorption-desorption guideline where sterilisation of soils is suggested to separate the processes of sorption and biodegradation (OECD 2000).

None of the methods successfully sterilised the soils and some changes in soils were identified pos*t*-treatment. Autoclaving destroyed the soil structure, turning it into a fine powder which would have an impact on the sorption behaviour of chemicals in any resultant test; consequently, it was not studied further. Sodium azide changed the pH of the loam soil suspension by 0.53 pH units, but not the sandy loam soil. Literature suggested that gamma irradiation was the most likely to sterilise the soils with the least amount of disturbance to its physico-chemical properties.

Experimental data concluded that gamma irradiation was probably the best method for sterilising soils for sorption-desorption experiments; however care needs to be taken to ensure that microbial activity is absent. The changes to soils after sterilisation varied depending on the individual soil properties, indicating that soils should be studied on a case-by-case basis.

3.2 Introduction

Active pharmaceutical ingredients (APIs) are ionisable compounds that can be lost from soil suspensions through sorption to soil particles, biodegradation, abiotic degradation (e.g. photodegradation), volatilisation and leaching into groundwater or other water sources (ECETOC 2013a; Lees et al. 2016). When undertaking environmental risk

assessments of chemicals in soil matrixes it can be difficult to distinguish the pathways of loss from suspension and ultimate fate of the chemical in the environment. To separate biodegradation from other loss mechanisms soil must be sterilised by appropriate chemical or physical processes (OECD 2000). The OECD 106 guideline does not define sterilisation or recommend a method to do it. The aim of sterilisation, as described in this chapter, is to remove or destroy all living bacteria and other microorganisms in soils (Oxford University Press 2002). Other methods have attempted to supress microbial activity which reduces the biodiversity of the soil fauna and keeps the microbial population stable throughout the length of experiments, such as the addition of sodium azide and mercury chloride.

When sterilising soils the soil physico-chemical characteristics must remain unchanged so that the results are comparable to those from non-sterile experiments. The important soil variables affecting interactions of soil with ionisable compounds include, pH, DOC, CEC, clay structure, ionic strength and particle size (Lees et al. 2016). A decision tree shows the pathways for identifying a suitable sterilisation method for an OECD 106 experiment (Figure 3.1).

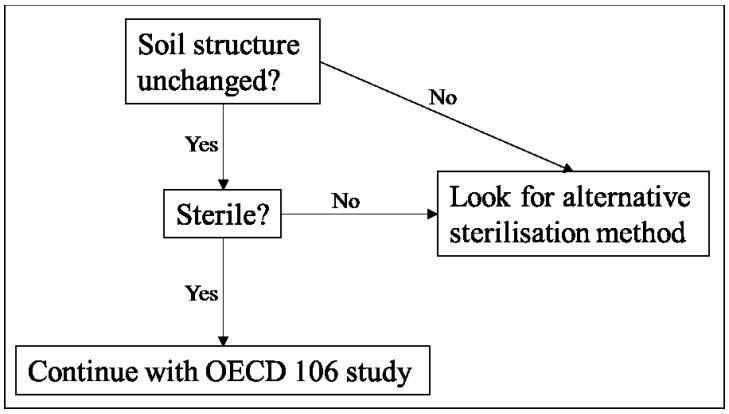


Figure 3.1 - Decision tree for finding the optimum method for sterilising soils for experiments following OECD 106

The methods of sterilisation discussed here are, autoclaving, gamma irradiation and addition of sodium azide to the soil. These treatments have been used to completely sterilise soils (autoclaving and gamma irradiation) (Redshaw et al. 2008; Xu et al. 2009b; Al-Rajab et al. 2010; Zhang et al. 2013) or to supress the microbial activity within a soil (sodium azide) (Lin et al. 2011; Zhang et al. 2013). Other sterilisation methods include using dry heat, microwave radiation and other chemical additions such as mercuric chloride or chloroform (Wolf et al. 1989; Trevors 1996). These methods were not used in the current study as literature has shown them not to be successful or use chemicals that are difficult or dangerous to handle and have consequently been banned from most applications (Wolf et al. 1989).

Two analytical techniques were used to estimate the extent of sterilisation; fluorescein diacetate (FDA) hydrolysis (Adam et al. 2001) and counting colony forming units on tryptone-glucose-yeast agar plates (Eaton et al. 1995).

The objectives of this study were to investigate the efficacy of common methods of soil sterilisation in reducing the microbial population, and how soil structure may be physically influenced by the process that may therefore impact sorption experiments described in the OECD 106 guideline.

3.3 Materials and specific methods

3.3.1 Soils

The soils used for this experiment were as described in the methods chapter, with the addition of a freshly collected soil (Table 3.1). The latter was analysed to compare FDA measurements on a soil that had been stored in the dark at room temperature for 2 years to a fresh soil sourced from Welltown near Kingston, Cornwall in July 2016 (named ‘Welltown’ soil here). This soil was chosen as it had recently been collected and

characterised when these experiment was being undertaken. All soils were air dried and sieved to <2 mm.

Table 3.1 - Selected soils properties (sandy loam and loam are mean values of different batch analyses ± S.D. (LUFA Speyer, 2015))

	Sandy loam	Loam	Welltown*
pH (10 mM $CaCl_2$)	5.7 ± 0.6	7.3 ± 0.1	4.4
Organic carbon (%)	0.67 ± 0.03	2.03 ± 0.22	4.94
Clay content (%)	6.3 ± 1.9	26.0 ±1.9	2.85 ± 0.09
Silt content (%)	33.8 ± 0.2	41.0 ± 1.4	72.96 ± 1.22
Sand content (%)	59.9 ± 1.9	33.0 ± 2.0	24.19 ± 1.30
Cation exchange capacity (MEQ $100g^{-1}$)	7.5 ± 0.9	33.0 ± 4.5	27.8

*Welltown soil properties provided by Anastasios Giallourou, SoGEES Plymouth University

3.3.2 Sterilisation methods

Three commonly reported sterilisation methods were compared in this study; autoclaving, gamma irradiation and sodium azide.

3.3.2.1 Autoclaving

Soils (6 ± 0.01 g) were autoclaved at 126 °C for 35 minutes under vacuum in polypropylene centrifuge tubes (Powlson et al. 1976). This process can be repeated with a room temperature incubation (approx. 24 hour) in-between autoclave cycles to ensure that all microbes and spores are thoroughly sterilised. The 24 hour delay allows hea*t*-resistant spores to germinate and then be killed on the next autoclave cycle (Miyaki et al. 1996).

3.3.2.2 Gamma irradiation

Soils were irradiated by a local company (BD Ltd, Plymouth UK). Subsamples of the soils were weighed into polyethylene sample bags (approximately 18 or 30 g depending on intended purpose) and double bagged. The dose provided to the soils was

25.6-26.1 kGy. Once the soils were returned to the laboratory they were handled cleanly under a laminar flow hood (Bassaire, class 100) to reduce contamination.

3.3.2.3 Sodium azide

Sodium azide was chosen to chemically inhibit microbial activity in soils due to its popularity in literature for use in pharmaceutical fate studies (Chefetz et al. 2006; Vasudevan et al. 2009; Lin et al. 2011). The concentration of sodium azide (Aldrich Chemicals Ltd or Acros Organics, UK) used was 0.2 g L^{-1} as this was shown to be effective in soil matrices (Yamamoto et al. 2009; Lin et al. 2011).

3.3.3 Sterility assessment

Two standard methods were employed to estimate the total enzyme activity and the quantity of colony forming units in the soils before and after each sterilisation treatment; fluorescein diacetate hydrolysis and counting colony forming units. These methods are estimates because of the diverse nature of microbial populations in soils meaning that not all microbial types will produce measurable effects (Bandick et al. 1999; Taylor et al. 2002). The scale of the estimates are unsure however as no data is available on accuracy of the estimates. In the data presented here comparisons are made with like-to-like soils so this will remove the majority of the uncertainty with these methods.

Fluorescein diacetate (FDA) is widely used to estimate total microbial activity in a range of environmental samples (Adam et al. 2001). The advantages of this method are the speed at which results can be obtained, its low cost and the reduced risk of contamination when compared with more traditional assays (Chand et al. 1994; Schumacher et al. 2015). The potential negatives of this method include the hydrolysis of FDA in the absence of live cells or quenching of the hydrolysed fluorescein by the matrix (Clarke et al. 2001).

Counting colony forming units is a common method of determining the quantity of viable microorganisms in soils that has been used for many years (Trevors 1996). The benefits of this method is that it is simple and requires very little equipment (e.g. usually just agar plates, incubator and a microscope) and it can also give an indication to the diversity of the microbial community (De Leij et al. 1994). The negatives associated with this method is that it usually relies on subjective counting by an individual laboratory analyst rather than an instrument, it is reliant on the extraction efficiency of removing the bacteria from soil particles and it also does not differentiate between living and dead cells so usually produces overestimates (Taylor et al. 2002).

3.3.3.1 Fluorescein diacetate hydrolysis

The method set out by Adam and Duncan (2001) was followed and adapted to optimise sensitivity by lengthening the incubation period. Colourless FDA is hydrolysed by a number of different cell-bound and free enzymes (e.g. proteases, lipases and esterases) providing a broad-spectrum indicator of soil biological activity (Bandick et al. 1999; Adam et al. 2001; Green et al. 2006). The hydrolysis releases a yellow-coloured end product, fluorescein, which is measured by spectrophotometry or UV-VIS (wavelength = 490 nm).

60 mM sodium phosphate buffer solution (pH 7.6)

A 120 mM buffer was prepared by dissolving 19.67 g sodium phosphate tribasic anhydrous (AlfaAesar, UK) in 1 L of HPW. Sodium phosphate monobasic dihydrate was added to achieve a pH of 7.6. The 60 mM buffer solution was prepared by diluting the 120 mM buffer using HPW and adjusting the pH as required with sodium phosphate monobasic dihydrate. Buffer solutions were stored at 4 °C for up to one week and the pH checked before use. A pH 7.6 buffer solution is used in all FDA hydrolysis experiments because FDA has been found to reach a maximum rate of hydrolysis at this pH (Green et

al. 2006). Using pH 7.6 also reduces the risk of solubilising organic matter that can interfere with the UV-VIS analysis and produce very high background blanks (Swisher et al. 1980; Adam et al. 2001).

Fluorescein diacetate solution (1000 µg FDA mL^{-1})

0.1 g fluorescein diacetate (AlfaAesar, UK) was dissolved in 100 mL AR grade acetone (Acros Organics, UK) and stored at 4 °C for up to one week.

Calibration solutions

Calibration solutions were prepared on the day of analysis using fluorescein sodium salt (Sigma Aldrich, UK) in 60 mM sodium phosphate buffer solution. Calibration graphs were prepared in the concentration range of 0-10 mg L^{-1} (Figure 3.2). Standard deviations were calculated but were too small to plot on the graph (Table 3.2)

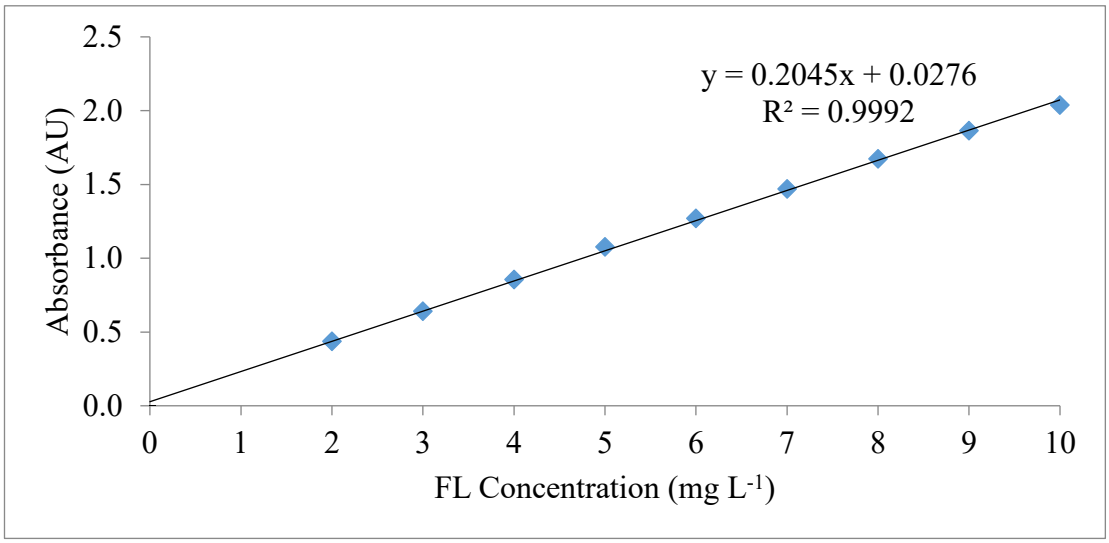


Figure 3.2 - Example calibration of fluorescein sodium salt by UV VIS spectrophotometry (λ=490 nm). Data were collected 01/09/2016

Table 3.2 - Mean and standard deviations of calibration presented in Figure 3.2 (n = 3)

Conc. (mg L^{-1})	2	3	4	5	6	7	8	9	10
Mean (AU)	0.436	0.640	0.855	1.077	1.270	1.468	1.672	1.864	2.037
S.D. (AU)	0.002	0.003	0.000	0.005	0.006	0.002	0.010	0.010	0.023

Procedure

The method outlined by Adam (2001) and Schofield (2015) was followed with the incubation time extended to maximise fluorescein production and make analytical measurements more robust. Soil (2 ± 0.01 g) was accurately weighed into sterile 50 mL polypropylene centrifuge tubes and 15 mL of 60 mM sodium phosphate buffer (pH 7.6) was added. A 200 µL aliquot of FDA (1000 µg FDA mL^{-1}) solution was added and the tubes were mixed by inversion. The tubes were incubated in a water bath at 30 °C for 3 hours, then centrifuged (2000 RPM, 5 minutes) and analysed as described. Samples were immediately analysed at 490 nm on a Hewlet*t*-Packard 3454 UV-VIS spectrophotometer. No termination step was used in this experiment as this may interfere with spectrophotometry by reducing the amount of fluorescein measurable (Adam et al. 2001; Schumacher et al. 2015). As a result, incubations were staggered to allow for immediate analysis once the incubation period was complete. Matrix blanks were included in the analysis with 200 µL of AR grade acetone used instead of FDA. These absorbance values were subtracted from sample data to account for matrix effects (Table 3.3).

Table 3.3 - Blank absorbance values compared to samples for FDA experiment

Soil	Mean absorbance for sample (AU)*	Mean absorbance for corresponding blank (AU)*
Loam	2.17 ± 0.07	0.32 ± 0.00
Irradiated loam	2.00 ± 0.05	0.35 ± 0.00
Sandy loam	1.03 ± 0.01	0.06 ± 0.00
Irradiated sandy loam	0.79 ± 0.02	0.05 ± 0.04
Welltown soil	2.30 ± 0.31	0.50 ± 0.00

*data as x□ ± S.D. n = 9 for data and 3 for blanks

Instrument limit of detection (LOD)

Instrumental LOD was estimated using Equation 3.1. The LOD of fluorescein was 0.3 mg L^{-1} and all sample concentrations were higher than this value before conversion to fluorescein production rate to take into account the incubation time.

3.1

$$\boldsymbol{LOD = mean\ of\ blank\ + (3 \times standard\ deviation\ of\ blanks)}$$

3.3.3.2 Estimation of colony forming units

The estimation of colony forming units was undertaken by the Matt Emery from the School of Biology (Plymouth, University). A representative soil slurry was decanted from tubes containing 1 : 5 soil : 10 mM $CaCl_2$ solutions, into sterile containers under a laminar flow hood. A 100 µL aliquot of a 1 : 10 dilution (using sterile water) was spread across the surface of a tryptone glucose yeast agar plate. Plates were incubated at 30 °C for 72 hours and colony forming units were counted.

Plating was used for soils containing sodium azide as this interfered with the FDA measurement. This was tested by adding 0.2 g L^{-1} of sodium azide to HPW and comparing

FDA results with pure HPW. HPW containing sodium azide had measured fluorescein concentrations three times higher than in just HPW (2.49 and 0.8 mg L^{-1} respectively).

3.3.4 DOC analysis

DOC was measured using a Shimadzu TOC (total organic carbon) 5000A analyser following the method described by Badr et al (2003) by Alan Tappin in the School of Geography, Earth and Environmental Science (Plymouth University, UK). Prior to analysis filtered samples (0.7 µm ashed glass fibre filters) were acidified using 6 M hydrochloric acid. HPW was used if samples required dilution.

DOC standards were made using potassium hydrogen phthalate with a concentration range of 0 – 677 µmol L^{-1} C. A straight line calibration graph was plotted and this was used to convert the peak area of samples into DOC concentration. A more detailed method can be found in the methods chapter.

3.3.5 pH analysis

pH measurement of all the soil treatments was measured on the same day. Soil (10 ± 0.01 g) and 25 mL 10 mM $CaCl_2$ was placed into polypropylene centrifuge tubes (50 mL; Fisher Scientific UK) in triplicate. Tubes were shaken for 15 minutes before pH was measured using a HANNA HI 9025 microcomputer pH meter fitted with a Camlab epoxy tough single junction combination pH electrode (Rowell 1994). This was calibrated using pH 4.01 and pH 7.00 buffers (Thermo Scientific). The pH reading was taken after the value had been stable for 10 seconds.

3.4 Results

3.4.1 Autoclaving

Autoclaving significantly changed the soil structure and visibly turned it into a powder increasing the surface area available for sorption of APIs. Measurement of the DOC concentrations in soil : water (1 : 5) showed that the loam soil had increased from 42.6 mg L^{-1} to 159.4 mg L^{-1} and the sandy loam increased from 8.7 to 47.1 mg L^{-1} (Figure 3.3). This meant that this method would not be appropriate for sorption experiments, as the soil could not be compared with non-sterile soils. Consequently, the sterility of the autoclaved soils was not measured.

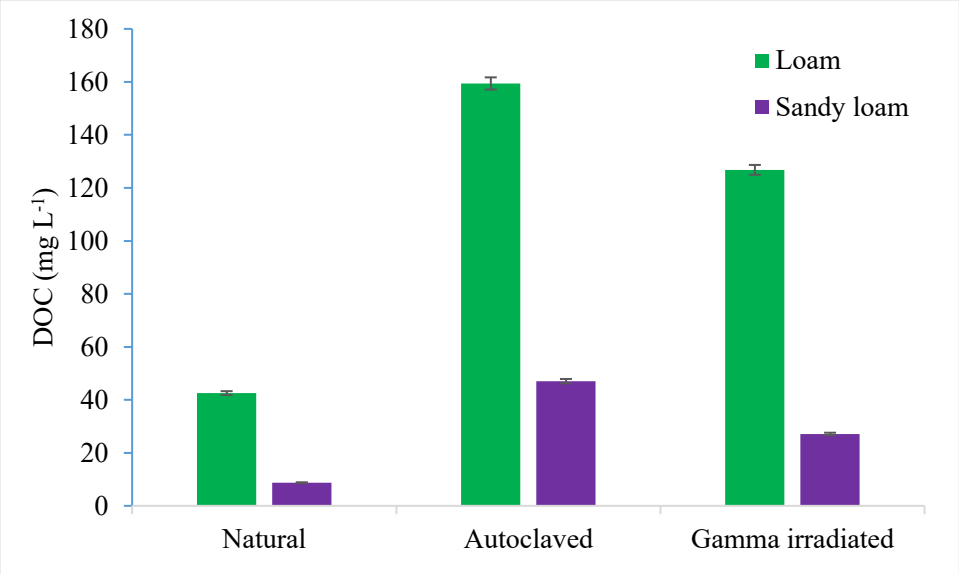


Figure 3.3 - DOC in treated soils ($\bar{x}$ ±S.D. n = 3)

3.4.2 Gamma irradiation

The DOC was impacted by the use of irradiation on the soils. In the loam soil the DOC increased from 42.6 to 126.8 mg L^{-1} and the sandy loam increased from 8.7 to 27.13 mg L^{-1} (Figure 3.3).

Gamma irradiation did not successfully sterilise the loam and sandy loam soils (Figure 3.4). A small but statistically significant decrease in the total soil enzyme activity was measured in both soils after gamma irradiation (*t*-test, unequal variances, two-tailed, $p \leq 0.05$). The soil sourced from Welltown was tested as it had not been stored for a long period of time (unlike the loam and sandy loam soils) so the total enzyme activity should not have been affected. Total enzyme activity in the fresh Welltown soil was not significantly different to the loam soil, which had been stored for 2 years (*t*-test, unequal variances, two-tailed, $p \leq 0.05$).

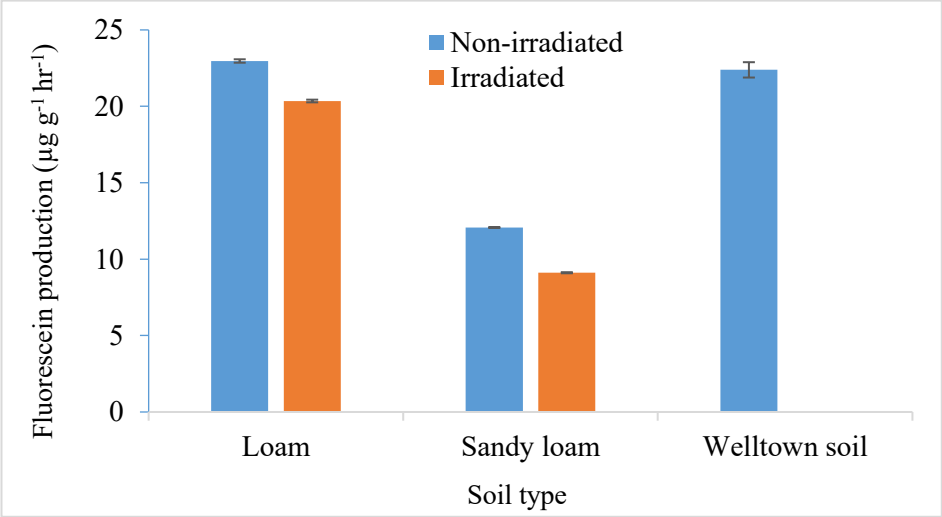


Figure 3.4 - Fluorescein production in irradiated and non-irradiated solid soil (presented as $\bar{x}$ ± S.D. n = 6 or 9)

3.4.3 Sodium azide

Samples were taken from the 120 hour API sorption experiment (i.e. included a mix of APIs) and colony forming units were counted after incubation on tryptone glucose yeast agar plates. Only one replicate was analysed for each time point due to time constraints in the School of Biology technical team. Sandy loam soil had numerous swarming colonies which made counting difficult so numbers presented in the graphs are approximate; however diversity was similar across untreated and treated soils and across

all time points. Loam samples showed a much different diversity depending on the treatment. Untreated loam soils contained swarming filamentous species (probably *Bacillus spp.*) whereas the treated soil did not, but had a number of colourful isolates instead. No overall microbial inhibition was observed after addition of 0.2 g L^{-1} sodium azide (Figure 3.5).

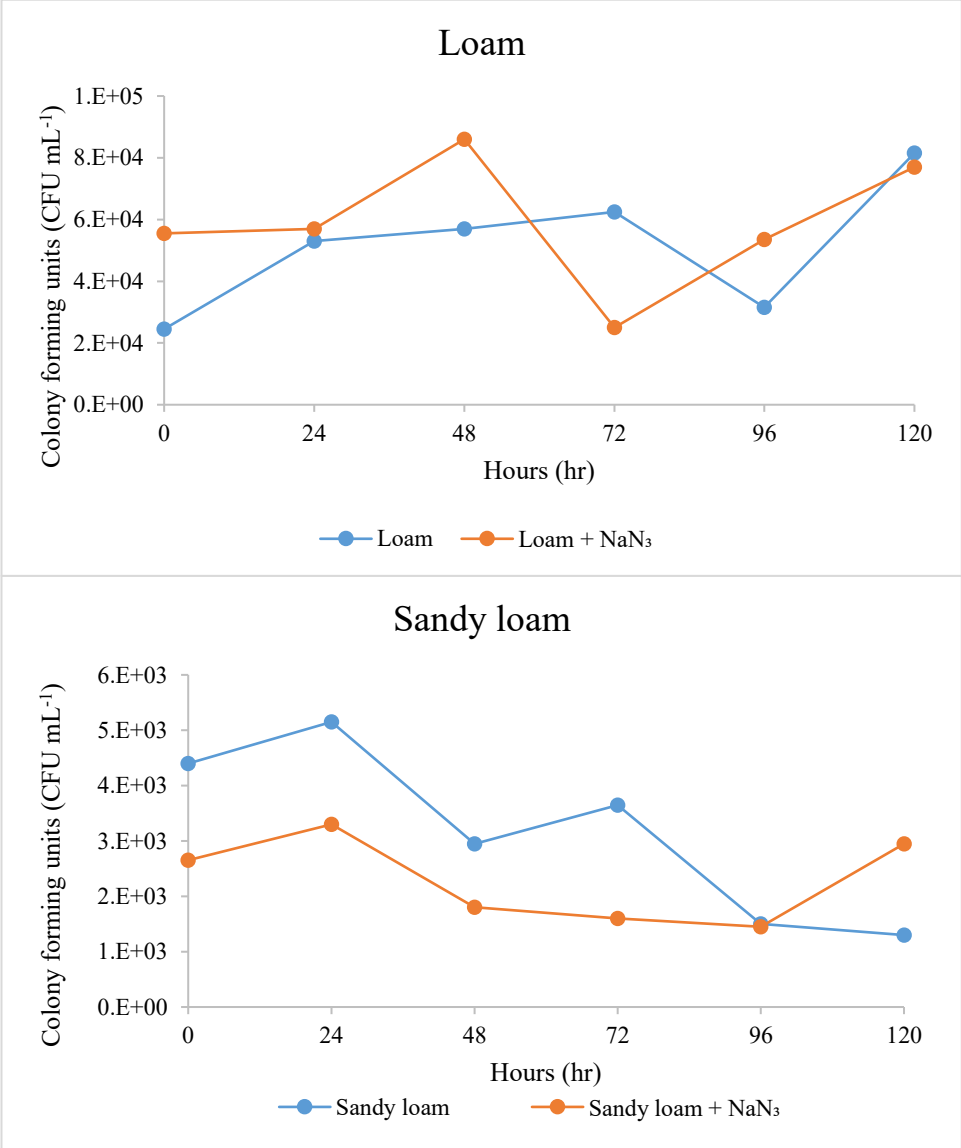


Figure 3.5 – Approximate colony forming unit count in soil suspensions treated with sodium azide (n = 1)

3.4.4 pH

A significant increase in soil suspension pH was identified in the loam soil (0.53 pH units) after the addition of 0.2 g L^{-1} sodium azide (Table 3.4) (*t*-test, unequal variances, two-tailed $p \leq 0.05$). No other treatments in the loam soil produced a significant differences compared to an unaltered 'normal' sample (*t*-test, unequal variances, two-tailed $p \leq 0.05$). Sandy loam produced a decrease in pH after all treatments of approximately 0.3 pH units that was significant in all cases (*t*-test, unequal variances, two-tailed $p \leq 0.05$).

Table 3.4 - pH of soil suspensions after sterilisation methods in 10 mM $CaCl_2$

	Loam*	**Sandy loam***
Normal	6.36 ± 0.05	5.83 ± 0.05
Autoclaved	6.44 ± 0.04	5.55 ± 0.01
Gamma irradiated	6.38 ± 0.11	5.53 ± 0.02
Sodium azide	6.93 ± 0.02	5.54 ± 0.01

*Data presented as $\bar{x}$ ± S.D. (n = 3)

3.5 Discussion

Microbial communities in soils vary considerably between soil samples, depending on many environmental factors, including soil moisture, aeration, land use, pH, temperature, organic matter and nutrient levels (van Elsas et al. 2006). Variations in microbial populations may lead to different rates of biodegradation of chemicals between soil types. This poses challenges for environmental risk assessments as separating biodegradation from sorption is vital for a robust risk assessment to be carried out. Thorough sterilisation of soils to be used in methods such as OECD 106 is needed to ensure that the processes can be investigated. To distinguish these two processes a sorption profile in sterile, or microbial-activity supressed, and natural soil needs to be

performed so that the physical-chemical structure of the soil is maintained in the sterilisation method.

Autoclaving is the most common sterilisation method for soils, due to the usual ease of access to an autoclave in many laboratories (Trevors 1996; Berns et al. 2008). It has been used in OECD 106 type research previously (Xu et al. 2009b; Zhang et al. 2013; Estevez et al. 2014; Mrozik et al. 2014). Autoclaving in the current study converted the soil to powder form and greatly increased the surface area available for sorption; this has been reported elsewhere (Trevors 1996; Berns et al. 2008). Berns et al. (2008) also observed a 29 to 37-fold increase in the DOC content of soil filtrates after autoclaving two soils. Large increases in DOC have been measured after autoclaving in other studies (Powlson et al. 1976; Shaw et al. 1999). Organic carbon physically trapped between particles may have been solubilised and autoclaving could also detach organic carbon from particle surfaces (Powlson et al. 1976; Berns et al. 2008). In contrast, another study measured a decrease in soil surface area (55 %) after one dry cycle (30 minutes at 121 °C) in the autoclave suggesting that soil pores may have collapsed and aggregation of clay size particles occurred, leading to a greater portion of sand size particles (Lotrario et al. 1995). A smaller decrease (40 %) in surface area was measured by Wolf et al (1989) after 2-3 cycles of autoclaving, which was attributed to the smoothing of irregular shaped particles and allowing clumping to take place. Differences in surface area after autoclaving could be attributed to different analytical methods of determining the aggregation of soil, such as mechanical or gentle aggregate fractionation (Berns et al. 2008). Autoclaving has also been shown to decrease soil pH attributed to the release of organic acids from the soil organic matter but other studies found no difference (Wolf et al. 1989; Shaw et al. 1999; Berns et al. 2008). Both of these results were measured in the data presented here; loam soil showed no difference in pH whereas sandy loam showed a decrease of 0.3 pH units (Table 3.4). The changes in the physical structure of the soils

observed in this and other studies indicate that autoclaving of soils will have an impact on the sorption profiles of APIs in soils, as increasing the surface area will increase available sorption sites. Furthermore, increasing DOC in sorption experiments can increase the sorption of analytes leading to inaccurate risk assessments (Day 1991; Carmosini et al. 2009). Impacts on soil seem to vary with different soils so individual assessments should be carried out when using autoclaving as a sterilisation method.

The data from FDA experiments was compared with literature data in non-irradiated soils (Table 3.5). Total enzyme activity from the FDA experiment in the sandy loam soil was lower than reported values, which may have resulted from the long storage period. The loam and Welltown soils showed similar total enzyme activity to the lowest reported data values (Table 3.5). Air drying soils reduces the concentration of adenosine 5′-triphosphate (ATP) which is used as a measure of microbial biomass in soil. Storing soils has been shown to decrease the ability of microbial biomass to restore the ATP concentration after rewetting (Mondini et al. 2002; De Nobili et al. 2006). For example a soil (from stubbed grassland) stored for 2 years had ATP concentrations of 14% that of the fresh soil after rewetting (De Nobili et al. 2006). Some soil microorganisms have developed capabilities of surviving in dry conditions for long periods to survive during drought periods such as endospores, cysts or conidia explain why some microbial activity can be recorded after periods of storage (Chen et al. 1973). The levels of organic matter probably has an impact on the survival of bacteria and soils with higher levels of organic matter 'protecting' microorganisms by reducing oxidative radical reactions (De Nobili et al. 2006). This is shown in Table 3.5 where the loam and Welltown soils have the highest enzyme activity and also have high organic carbon levels (Table 3.1).

Table 3.5 - Comparison of experimental total enzyme activity data to literature values with associated soil properties

Land use/soil type	pH	Organic carbon (%)	Cation exchange capacity (MEQ $100g^{-1}$)	Fluorescein production ($\mu g\ g^{-1}\ hr^{-1}$)	Reference
Loam	7.3 ± 0.1	2.03 ± 0.22	33.0 ± 4.5	23.0±0.1	This study
Irradiated loam				20.3±0.1	This study
Sandy loam	5.7 ± 0.6	0.67 ± 0.03	7.5 ± 0.9	12.1±0.01	This study
Irradiated sandy loam	5.7 ± 0.6			9.1±0.04	This study
Welltown soil	4.4	4.94	27.8	22.4±0.5	This study
Crop land silty clay loam				~60	(Schumacher et al. 2015)
Crop land loam				~28	(Schumacher et al. 2015)
Crop land loam				~40	(Schumacher et al. 2015)
Grassland sandy loam				~22	(Schumacher et al. 2015)
Crop land sandy loam				40	(Debosz et al. 2002)

A small, but statistically significant, change in total enzyme activity was measured after gamma irradiation of the soils. This may be because the amount of radiation used was too low (25 kGy), although this level of radiation has been successful in several studies (Lensi et al. 1991; Bank et al. 2008; Buchan et al. 2012). Others have suggested that a higher radiation dose is required to achieve sterilisation (up to 70 kGy) (McNamara et al. 2003; Kahle et al. 2007). Higher doses have been reported to affect soil physical-chemical properties such as variations in soluble carbon, exchangeable cation concentrations, pH and clay mineral chemistry (Lensi et al. 1991).

Gamma irradiation (25 kGy) of soils has been shown to produce a 1.7 to 3.3 fold increase in DOC, while preserving the mineral carbon content in the soils (Lensi et al. 1991). Smaller increases in DOC were measured in soils irradiated at 35 kGy where 2 % of total organic carbon being released into solution (Berns et al. 2008). It was hypothesized that increases in DOC after irradiation was probably due to lysis of cells and degradation of soil organic matter (Lensi et al. 1991). An increase in clay fraction and a decrease in the sil*t*-sized aggregate fraction suggesting that irradiation broke down a portion of soil aggregates and produced more clay size fraction from silt; this was more obvious in autoclaved soil than gamma irradiated (Berns et al. 2008). There are no consistent trends apparent in studies reporting effects of irradiation on pH; however some have suggested that the moisture content of soil at the time of irradiation may change soil pH (Lotrario et al. 1995; McNamara et al. 2003). CEC has been measured to decrease in soils after irradiation (20 kGy) from 39 to 31 $cmol_c\ kg^{-1}$ and was attributed to the breakdown of natural organic matter (Bank et al. 2008). Changes in CEC will impact the sorption of ionisable compounds to soil depending on the charge on the compound and whether there is an increase or decrease in CEC. Decreases in CEC with positively charged compounds will cause a decrease in sorption due to a reduction of potential sorption sites. Other studies have also found that all studied sterilisation methods (irradiation, autoclaving and sodium azide) had no significant effect on CEC (Wolf et al. 1989; Lotrario et al. 1995). The variation presented in the literature with regards to changes in soil texture and chemistry after gamma irradiation suggests that, while this may be the best available method of soil sterilisation for sorption studies, the reality is that different soils and the amount of gamma irradiation used will have varying results. When irradiation is used, controls must be in place to limit changes to soils so that sterilised soils can be compared with natural soils; for example, by comparing soil physico-chemical properties before and after irradiation. Gamma irradiation is usually

carried out at specialised facilities so problems can occur if samples are contaminated after irradiation, also this adds delays and additional costs to research.

Sodium azide was not successful in inhibiting microbial activity in the soils at the concentration used in this experiment. This concentration (0.2 g L^{-1}) has been used in sorption experiments as a biocide to minimise or suppress microbial activity (Yamamoto et al. 2009; Lin et al. 2011). Higher concentrations of sodium azide have also been used ranging from 0.5 to 0.98 g L^{-1} (ter Laak et al. 2006a; Vasudevan et al. 2009; Zhang et al. 2013). The lower end of reported concentrations was used in the present study as sodium azide has the potential to interfere with soil chemical properties (Trevors 1996). Soil suspension pH increased slightly after the addition of sodium azide in the loam soil (Table 3.4). A more significant pH change has also been identified in the literature changing from pH 5.2 to 8.7 after 30 days incubation with 5 % sodium azide compared with control samples where no change occurred (Rozycki et al. 1981).Variation of pH will be a function of the soil buffering capacity (Trevors 1996). This could potentially influence the ionisation state of APIs or other chemicals that are in ionic form at environmental pHs. Sodium azide is low cost and easy to access but it is toxic so must be handled and disposed of with care.

3.6 Conclusion

None of the samples in this study were successfully sterilised so the OECD 106 method could not be completed in sterile soils, separating sorption from biodegradation. As reported sterilisation methods were followed in this study, this has interesting implications for future research. Sterilisation techniques may be soil specific and should be thoroughly tested prior to undertaking abiotic sorption experiments for environmental risk assessments.

All of the methods presented here can influence soil physical-chemical properties; this could lead to incomparable sterile sorption profiles making the data of less value. Recommendations for sterilising soils for sorption-desorption batch experiments should be included in the guidelines documentation. This will aid future research and changes to soils should be kept to a minimum. The difficulty with this recommendation is that the different soils appear to act differently under different sterilisation conditions, with parameters such as the pH in the loam soil increasing after sodium azide treatment, while no change occurred for the sandy loam soil. Additionally differences in DOC were measured after autoclaving and gamma irradiation.

Having compared three widely used sterilisation approaches in this study it appears that gamma irradiation is more appropriate for the OECD 106 method as it has the lowest impact on the soil structure, though care needs to be taken to ensure that sterilisation is achieved.

4 Sorption of pharmaceuticals to soil in suspension

4.1 Overview

This chapter investigates the sorption of four APIs in two soil suspensions. This work was undertaken to obtain data on the fate of APIs in soil without SWW addition. The aim of the study was to use parts of the OECD 106 method to assess the sorption of API behaviours in soil suspensions and identify physico-chemical mechanisms controlling the partitioning processes.

The four APIs studied behaved differently in the non-sterile soils. Ofloxacin was characterised by a rapid and mostly irreversible loss from both soil suspensions. Propranolol showed a similar loss from suspension as ofloxacin but the overall loss from suspension was less and had some potential for desorption Naproxen and nevirapine acted similarly with very little loss from both soil suspensions over the length of the experiment, which was partially reversible. The overall driver of the differences between the soils was the ionisation state of the APIs, which was dependent on soil pH. Organic matter in soils is one of the larger drivers controlling the fate of APIs in soils, but due to a lack of consistency in the Log K_{oc} data between the two soils, there must be another important sorption site that should be included as well. Clay and other mineral sites have been proposed in literature as having the potential to interact with ionised chemicals (Vasudevan et al. 2009; Yan et al. 2012). The importance of clay sorption depends on the environmental pH, API pK_a and anion : cation exchange capacity ratio (Lees et al. 2016).

The overall conclusion of this research was that the current driver for terrestrial ERA, namely Log K_{oc} does not fully take into account all of the drivers that control the fate of APIs in soils. As Log K_{oc} is the trigger value for an in depth extended ERA focussed on the terrestrial compartment, this value needs reassessing for ionisable compounds such as APIs.

4.2 Introduction

APIs are introduced to the soil environment through sewage sludge addition as a source of nutrients, animal husbandry practices and wastewater irrigation. There has been a global increase in the use of APIs in recent decades due to population growth, increasing affluence, changes in disease burdens and easier access to medication (Lees et al. 2016). The increase of APIs entering the environment has raised the need for robust environmental risk assessments to be developed for these chemicals. It is only since 2006 that environmental risk assessments (ERAs) have been required for all new marketing authorisation applications for human medicinal products in the European Union (EMEA 2006). The underlying assumption for the ERA is that wastewater is universally treated in sewage treatment plants (as required under EU law) which, as has been described, is not the case for all countries. Furthermore, the European ERA is only concerned with exposure to API following application of sewage biosolids to soil, and does not include additional scenarios, such as irrigation with wastewater and other contaminated water sources. The appropriateness of some of the physico-chemical based action limits in the development of more robust terrestrial ERAs for APIs in the EU are also under scrutiny (such as log K_{oc}), primarily because the majority of APIs (> 80 %) can exist either as cations, anions or zwitterions within the pH range covering most surface waters (ECETOC 2013a).

Loss of APIs from a soil suspension is the result of three main factors; sorption to soil solids, biodegradation and filtration through the soil profile (Chefetz et al. 2008; Lin et al. 2011). To assess this loss by sorption, the Organisation for Economic Co-operation and Development (OECD) has developed a test guideline to measure the adsorption-desorption of chemicals in a variety of soils using a batch-equilibrium method. The OECD guidelines are internationally accepted standard methods and are used by governments, industry and independent laboratories to determine the potential hazards

associated with a chemical (OECD 2016). OECD 106 is a test guideline aimed at estimating the sorption and desorption of a chemical in different soils. The main principle of this method is that a known concentration of the test substance is spiked into a known weight of soil in a 10 mM $CaCl_2$ solution. This solution is used to minimise changes in ionic strength of the adsorption isotherms by acting as a background electrolyte (EPA 1992). The mixture is then shaken for a set time, centrifuged and filtered when required prior to analysis. It is an indirect method as the aqueous phase is analysed and the difference between the amount of test substance added and recovered from the liquid phase is assumed to be the amount adsorbed to the soil after taking into account any potential loss to apparatus. Some loss from suspension could potentially be attributed to biodegradation, as the experiment was not sterile due to the problems associated with creating a comparable sterile soil (Chapter 3). A sorption value normalised for soil organic carbon (Log K_{oc}) is then obtained which can be used to predict partitioning in soils with different physico-chemical characteristics (OECD 2000).

The OECD 106 guideline was selected for this study as it has been used in both industrial and research contexts for assessing the potential environmental impact of APIs in soil (Drillia et al. 2005; Mrozik et al. 2014; Peruchi et al. 2015). It also covers soil types expected in areas that rely on wastewater for irrigation due to water shortages. Finally, the use of a standard method allowed for more direct comparisons with published data.

4.3 Materials

4.3.1 API information

APIs were selected as described in Chapter 2. Briefly four APIs were chosen for this research: ofloxacin, propranolol, naproxen and nevirapine. These cover a range of treatment areas and are used widely in areas where wastewater irrigation occurs.

Additionally, these APIs have a wide range of physico-chemical properties, particularly the charge associated with the molecule at the test pH. To estimate what fraction of the API will be ionised at given experimental pH using the pK_a of the compound, Equation 4.1 was used (ECETOC 2013a). This shows a range of ionisation states for each API during these experiments (Figure 4.1). Nevirapine does not have a negative charge at soil pH levels, as would be expected if the pK_a value (2.8) alone was used to estimate it. Nevirapine will be neutral at the experimental pH and becomes protonated at low pH values near the pK_a, this was deduced by using the structure of the molecule (Chapter 2).

4.1

$$\propto = \frac{\mathbf{1}}{\mathbf{1} + \mathbf{10}^{\boldsymbol{A(pH-pKa)}}}$$

Where α = fraction ionised and A is +1 for acids or -1 for bases

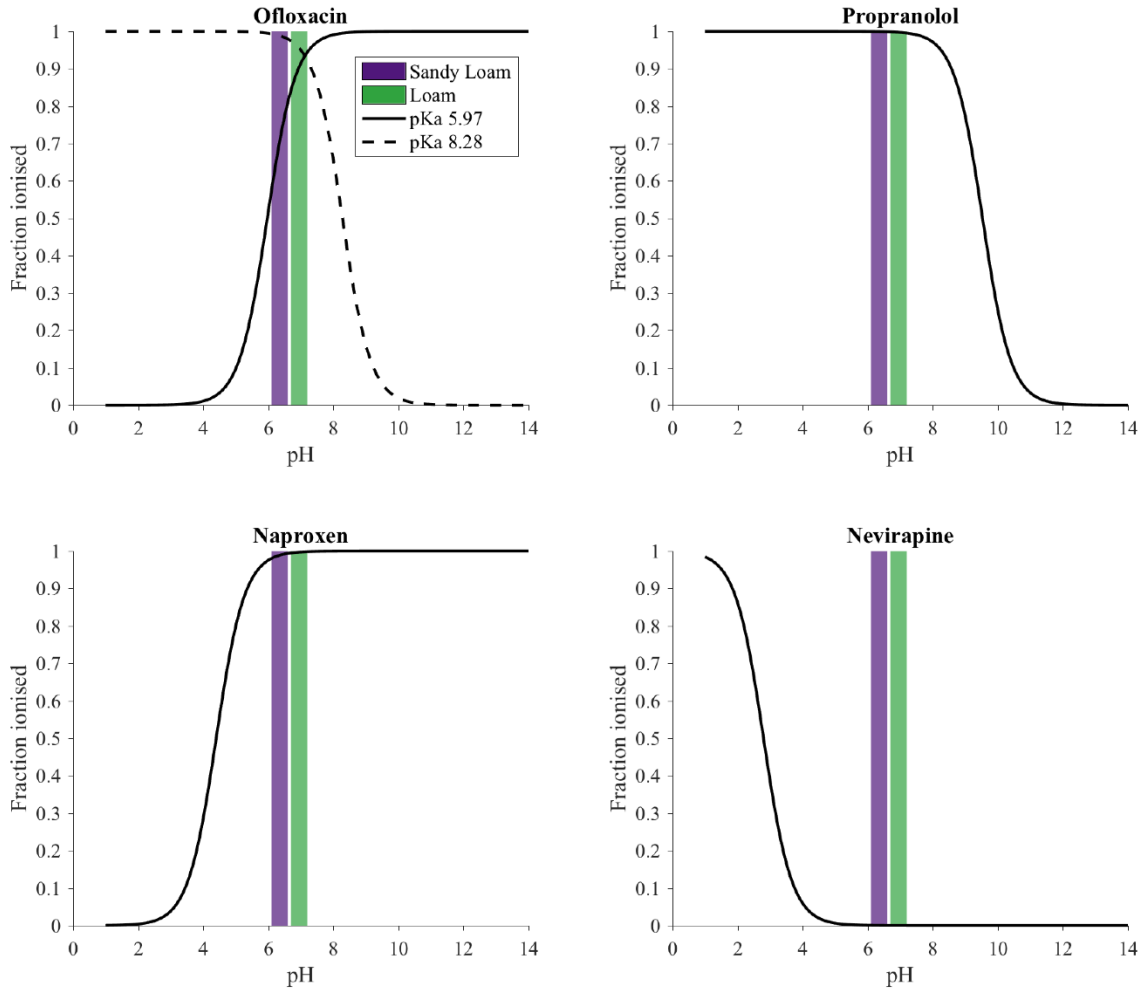


Figure 4.1 – pH dependent ionisation of APIs studied. Purple bar shows range of pH in sandy loam and green bar shows loam range.

4.3.2 Soils

Two soils were purchased from Lufa Speyer (Germany). They had at least a 5-year history of no pesticide, biocidal fertiliser, or organic manure application, resulting in a soil that was as 'clean' from contaminants as possible. The soils were air-dried and sieved to < 2 mm. More detail on the soils can be found in Table 2.10.

4.4 Methods

The method was developed following the OECD 106 guideline and adapting to suit the research needs (OECD 2000). A preliminary study was undertaken in order to determine the stability of APIs through the test period, and to determine a suitable soil : solution ratio and spiking concentration for the sorption kinetics experiment.

4.4.1 Soil : solution ratio selection

The OECD 106 guideline sets out several parameters when selecting the soil : 10 mM $CaCl_2$ solution ratio including using at least 1 g of soil, achieving preferably > 50 % sorption of the test substance to the soil; the ratio can range from 1:1 (low K_d) to 1:100 (high K_d) depending on the estimated K_d of the API (OECD 2000). After estimating the K_d of the APIs or using published values or modelled data, a suitable ratio could be decided upon with reference to a relationship graph (Figure 4.2). This was not possible for nevirapine as K_d data was not available. The estimated soil filtrate concentration at equilibrium for all APIs was above the analytical method's LOD and LOQ (Table 4.2).

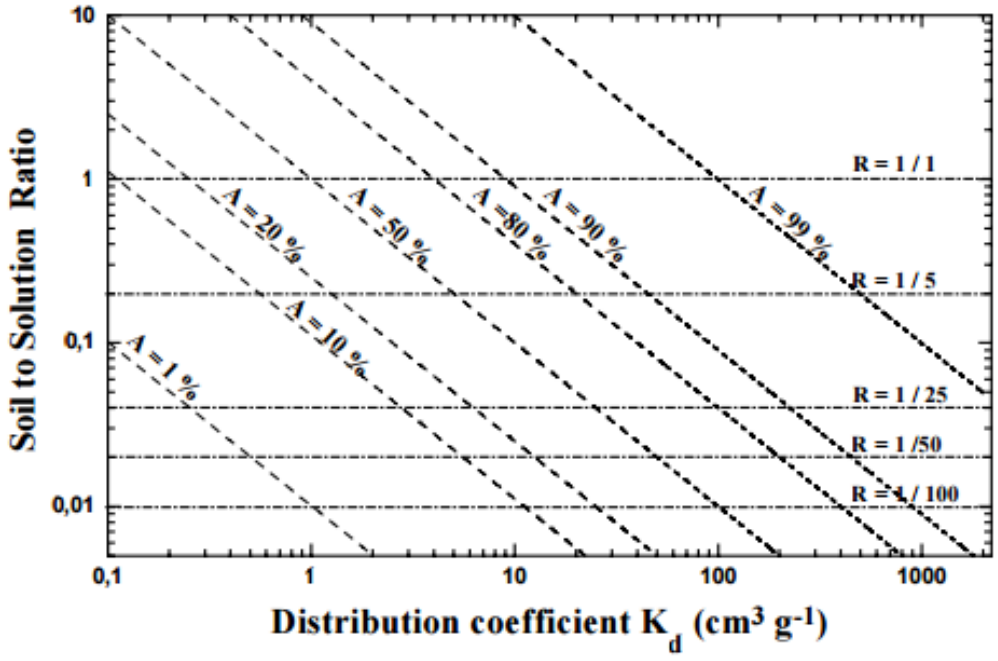


Figure 4.2 - Relationship between soil : solution ratios and K_d at various percentages of adsorbed test substance; A = adsorption, R = soil : solution ratio (OECD 2000).

A 1 : 5 weight to volume ratio (6 g soil to 30 mL 10 mM $CaCl_2$) was chosen as the best fit for all APIs' K_d values following the OECD guidelines. If performing this experiment on individual APIs a different ratio would have been chosen for ofloxacin as its large K_d (309-3554, Chapter 2) resulted in very low concentrations in the liquid phase, even at higher concentrations of the API.

Table 4.1 - Predicted percentage adsorption of APIs at a 1 : 5 soil : solution ratio using published* K_d values for the APIs

API	Low K_d predicted adsorption (%)	High K_d predicted adsorption (%)
Ofloxacin	97	<99
Propranolol	80	<99
Naproxen	20	80

*(Nowara et al. 1997; Drillia et al. 2005; Xu et al. 2009b; Yamamoto et al. 2009)

Table 4.2 - Predicted filtrate concentrations calculated from K_d

API	Concentration added (µg L^{-1})	Predicted filtrate concentration range at sorption equilibrium (µg L^{-1})
Ofloxacin	500	5.0 – 15.0
Propranolol	240	2.4 – 48.0
Naproxen	58	11.6 - 46.4
Nevirapine	50	No K_d was available but this API was predicted to have little sorption to soils as it will be mostly unionised at experimental pH

Preliminary experiments were performed on all APIs in 1 : 5 (w/v) soil suspensions shaken for four hours to ensure this ratio was correct and that the API concentrations added were adequate (Table 4.2). Propranolol, naproxen and ofloxacin were measured by fluorescence spectroscopy to achieve rapid analysis and to minimise the generation of waste solvent. As nevirapine was added to the suite of studied APIs at a later stage, and cannot be detected by fluorescence spectroscopy, it was analysed by HPLC-HRAM-MS using the method developed for this study.

The results show that the losses from suspension after shaking for four hours were quantifiable, especially on the HPLC-HRAM-MS which has lower LODs/LOQs for all APIs (Table 4.3). As expected, propranolol and ofloxacin had high losses from suspension. Ofloxacin produced very high loss from the liquid phase but the concentration was at least three times as high higher than LOQ in the relative matrix so this concentration was used rather than spike the experiment even higher. Naproxen and nevirapine showed low losses from soil suspensions in comparison to the other APIs. Nevirapine showed no significant change within the analytical accuracy of the technique (Chapter 2).

Table 4.3 - Preliminary loss of API from suspension after shaking for 4 hours

API	Loss from loam suspension (%)	Loss from sandy loam suspension (%)
Ofloxacin	99.7	99.4
Propranolol	95.3	74.8
Naproxen	58.1	28.9
Nevirapine	-0.6	-16.1

4.4.2 API loss from 10 mM $CaCl_2$ solution to soil

A 6 g aliquot of non-sterile soil was added to 50 mL polypropylene centrifuge tubes along with 30 mL of 10 mM $CaCl_2$ solution in triplicate for each time point. A set of solution blanks were produced with 30 mL 10 mM $CaCl_2$ in centrifuge tubes without soil. These were all put onto a reciprocal shaker (132 rpm), laid horizontally to allow the system to equilibrate overnight. Subsequently, ofloxacin (500 µg L^{-1}), propranolol (200 µg L^{-1}), naproxen (30 µg L^{-1}) and nevirapine (50 µg L^{-1}) were added to the tubes, which were then returned to the shaker. Tubes were sacrificed at pre-selected times after API addition (0, 3, 6, 24, 48, 72, 96, 120 hours), centrifuged (4000 RPM, 15 minutes) then filtered using 0.7 µm glass fibre filters (Fisher Scientific, UK). The solution control tubes (no soil) were shaken for a total of 189 hours to include measurement of potential degradation during the desorption experiment (Section 4.4.4). Samples were then stored at -20 °C until analysis, along with calibration solutions and blank soil suspensions prepared in parallel. Analysis of the soil filtrates was performed by HPLC-HRAM-MS using the method described in Chapter 2. This experiment was carried out at room temperature (20 - 25 °C) and in the dark (tubes wrapped in aluminium foil) to ensure that photodegradation of the APIs did not occur.

All the APIs were spiked into soil together rather than in individual experiments. This was due to two reasons; firstly, it reduced the overall experimental time and reduced the quantity of soils and solvents required for analysis. This led to more experiments such as the desorption and isotherms being able to be completed with the limited time available on the HPLC-HRAM-MS. Secondly in a real world scenario APIs would be found in mixtures, not just with other APIs but with a vast array or organic and non-organic compounds that may impact sorption fate in soils (Kočárek et al. 2016). There may be some risk that the sorption data gathered from these experiments could be impacted by the combined spike rather than just the soil parameters (Kočárek et al. 2016).

Two soil tests were undertaken in parallel to understand if changes in the soil suspensions could have influenced the sorption results. pH, DOC and the overall charge on soils were measured at all the sampling time points in centrifuge tubes without APIs. The methods followed are described in Chapter 2.

The concentrations measured in the soil filtrates were used to calculate the K_d, Log K_{oc} and percentage sorption for each API, assuming that all removal of APIs occurred through sorption to the soil, after correction for loss to filter papers.

The distribution coefficient, K_d, is the ratio of the masses of the API in the soil and in the aqueous phase at equilibrium (Equation 4.2) (OECD 2000).

4.2

$$K_d = \frac{m_s\,(eq)}{m_{aq}\,(eq)} \cdot \frac{V_0}{m_{soil}}$$

Where, K_d = distribution coefficient (mL g^{-1}), m_s (eq) = mass of API sorbed to soil at equilibrium (µg), m_{aq} (eq) = mass of API in aqueous phase at equilibrium (µg), V_0 = initial volume of aqueous phase (mL), m_{soil} = dry mass of soil (g)

Calculating the organic carbon-normalized adsorption coefficient, K_{oc}, allows for comparisons between soils with different OC contents (Equation 4.3).

4.3

$$K_{oc} = K_d \cdot \frac{100}{\%\ OC}$$

Where, K_{oc} = organic carbon normalized adsorption coefficient, (mL g OC^{-1}), % OC = organic carbon content in soil (%)

The percentage adsorption was used to calculate the percentage of API sorbed to the soil related to the quantity present at the start of the experiment (Equation 4.4).

4.4

$$A_{ti} = \frac{m_s \cdot 100}{m_0}$$

Where, A_{ti} = adsorption percentage at the time point t_i (%), m_0 = mass of API at the start of experiment (µg)

The operational definition of equilibrium is the minimum amount of time required to establish a rate of change of the solution concentration of ≤ 5 % per 24 hour interval (Equation 4.5) (EPA 1992). This allows from some flexibility in equilibrium when processes other than sorption take over dominance in the system, such as degradation.

4.5

$$\frac{\Delta C}{\Delta t} \leq 0.05\ per\ 24\ hour\ interval$$

Where, ΔC = change in concentration (µg L^{-1}) and Δt = change in time (hrs)

4.4.3 Sorption isotherms

The soil : solution ratio (1 : 5) used for the 120 hour experiment was maintained but the concentration of APIs was varied (Table 4.4). Mixtures were allowed to equilibrate overnight in the dark on a shaker before being amended with a range of five

API concentrations (OECD 2000). Naproxen and nevirapine were spiked together into the same soils to reduce quantity of samples for HPLC-HRAM-MS whereas propranolol and ofloxacin were done separately. These were then returned to the shaker in the dark and then agitated until adsorption equilibrium had been reached from the 120 hour experiment (Table 4.6). These samples were then centrifuged, filtered and stored at -20 °C before analysis.

Some of the concentrations of APIs chosen are mostly higher than those found in the natural soil environment (Chapter 1) (Table 4.4). High concentrations were chosen for ofloxacin as preliminary experiments showed that the sorption of this API to soil is very high (Table 4.3). This range of concentrations was needed in this case to ensure results were greater than the LOQ. The OECD 106 guideline states that for calculating isotherms concentrations should differ by two orders of magnitude, which is the case for propranolol, naproxen and nevirapine. This was not possible for ofloxacin as the spike concentration needed would have been higher than the solubility or below LOQ.

Table 4.4 - Concentrations of APIs used for sorption isotherms (µg L^{-1})

Ofloxacin	Propranolol	Naproxen	Nevirapine
500	2	5	5
1000	20	20	20
2000	100	50	50
2500	150	100	100
5000	200	150	150

The data from these experiments was then fitted to Freundlich or Langmuir isotherms. The Freundlich isotherm describes reversible adsorption that is not restricted to the formation of a monolayer of API on the soil surface (Foo et al. 2010). It is widely used in applications such as organic compounds adsorbing onto activated charcoal and

has be widely applied to APIs in soils (Foo et al. 2010; Kim et al. 2012; Yu et al. 2013; Peruchi et al. 2015). The Freundlich isotherm relates the amount of test substance absorbed to the concentration of the test substance in the liquid phase at equilibrium (Equation4.6) (OECD 2000). The higher the K_F the higher the maximum sorption capacity. 1/n is a measure of the linearity of adsorption, giving information on sorption sites and potential competition with water molecules. When 1/n < 1 the mobility of chemicals at very high concentrations will be underestimated by K_d or K_{oc} measurements made at low concentrations (ECETOC 2013a).

4.6

$$q_e = K_F C_e^{1/n}$$

Where, q_e = concentration of API adsorbed to soil at equilibrium (µg g^{-1}), K_F = Freundlich adsorption coefficient ($mL^{1/n}$ $\mu g^{(1-1/n)}$ g^{-1}) , C_e = concentration of API remaining in the liquid phase at equilibrium (μg^{-1} mL), n = regression constant

The Freundlich isotherm can be written as a linear equation to be easily solved (Equation 4.7).

4.7

$$log q_e = log K_F + \frac{1}{n} log C_e$$

The Langmuir isotherm assumes a single layer adsorption on an energetically homogenous surface which has a finite sorption capacity, it was originally developed to describe gas-solid phase adsorption onto activated sorption (Langmuir 1916). This isotherm refers to homogeneous adsorption where each molecule has constant enthalpies and sorption activation energy (Foo et al. 2010). It can be used to estimate the bonding energy (K_L) (L μg^{-1}) and the maximum possible adsorption possible onto a surface (M) (µg g^{-1}) (Equation 4.8) (EPA 1992).

4.8

$$q_e = \frac{K_L M C_e}{1 + K_L C_e}$$

The Langmuir equation can be solved in linear format (Equation 4.9).

4.9

$$\frac{C_e}{q_e} = \frac{1}{K_L M} + \frac{C_e}{M}$$

4.4.4 Desorption

Desorption of APIs after sorption equilibrium had been reached provided information on sorption strength. This is an important feature as it can provide ERAs with more data on the overall fate and mobility of an API in soils, especially those in wet climates or with repeated wastewater irrigation schemes. This experiment provides data on how persistent an API might be in soils and therefore indicates whether it is a greater risk in the soil compartment or if it can move through the soil profile to enter groundwater.

The OECD 106 parallel method was followed where samples are sacrificed at each time point (OECD 2000). Soils were prepared as detailed in section 4.4.2 and spiked with the same concentration of APIs after the system had been shaken overnight. After addition the tubes were shaken in the dark at room temperature (18 - 22 °C) until adsorption equilibrium was reached (Table 4.6). The tubes were centrifuged (4000 RPM, 15 mins) and the supernatant removed, then a subsample was filtered for analysis by HPLC-MS. The exact volume of 10 mM $CaCl_2$ extractant was then replaced and the soil pellet resuspended for 5 seconds on a minishaker. Samples were sacrificed in triplicate at 0, 3, 6, 24, 48, 72, 96 and 120 hours, centrifuged and filtered as stated then stored at -20 °C until analysis, along with matching calibration solutions. To identify changes in the

soil suspensions after 10 mM $CaCl_2$ replacement pH and DOC was measured from separate tubes handled at the same time but without APIs in them.

Desorption was calculated as the percentage of the test substance desorbed and related to the quantity of substance previously adsorbed at desorption equilbrium, taking into account the incomplete removal of the supernatant after the sorption experiment (approximately 3 mL) (Equation 4.10) (OECD 2000).

4.10

$$D = \frac{m_{des\ aq}}{m_{ads\ s}} \cdot 100$$

Where, D = percentage desorbed (%), $m_{des\ aq}$ = mass API desorbed from soil (µg), m_{ads} s = mass of API adsorbed onto soil at adsorption equilbrium (µg)

The apparent desorption coefficient (K_{des}) is the ratio between the mass of the API on the soil and the mass concentration of the desorbed API in the aqueous solution, once desorption equilibrium has been reached (Equation 4.11) (OECD 2000).

4.11

$$K_{des} = \frac{m_{ads\ s} - m_{des\ aq}}{m_{des\ aq}} \frac{V_T}{m_{soil}}$$

Where, K_{des} = desorption coefficient (mL g^{-1}), V_T = volume of aqueous phase (mL), m_{soil} = mass soil (g)

4.4.5 Data analysis

Data was analysed in a variety of ways depending on the requirements of the experiment.

Grubbs' test was used to identify outliers in all data (Miller et al. 2005). It compares the deviation of the suspect value from the sample mean with the standard deviation of the sample (Equation 4.12). Once G had been calculated for suspect values

it was compared with the critical value for G (p=0.05) (Table 4.5). If the calculated value of G was higher than the critical value that suspect value was defined as an outlier and removed from any future calculations.

4.12

$$G = \frac{(suspect\ value - \bar{x})}{s}$$

Where, G = Grubbs' test statistic, $\bar{x}$ = mean including the suspect value, s = standard deviation including the suspect value

Table 4.5 - Critical values of G (p = 0.05) (Miller et al. 2005)

Samples size	Critical value
3	1.155
4	1.481
5	1.715
6	1.887

Where significant differences in two sets of data were assessed, a two-sample *t*-test (unequal variance) was performed at the relevant significance level (p = 0.01 or 0.05) (Equation 4.13) (Miller et al. 2005). The value was compared to a table of critical values and was significant when it was greater than the critical value (Miller et al. 2005).

4.13

$$t = \frac{(\overline{x_1} - \overline{x_2})}{s\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

Where, t = *t*-statistic, $\bar{x}$ = mean, s = standard deviation, n = number samples

4.4.5.1 Modelling of sorption

The Franco and Trapp (2008) model was used to estimate Log K_{oc} for the four APIs in this study (Section 1.3.2). This model initially uses the Henderson-Hasslebalch

equation to estimates the speciation of monovalent and amphoteric ionic compounds (Equation 4.14 and 4.15). Amphoteric compounds are those that are able to react as both and acid and a base and will exist mostly as zwitterions in a certain range of pH. It then uses the amount ionised to estimate Log K_{oc} along with some other parameters (Equation 4.16 for acidic APIs and 4.17 for basic APIs). For acidic compounds only the pH can be varied in the equations to predict the effects of pH on sorption (Franco et al. 2009). If speciation of amphoters is calculated according to equation 4.15 it is possible to sum the contribution of the sorption of the anionic, cationic and neutral fraction using equations 4.18.

4.14

$$\phi_n = \frac{1}{1 + 10^{a(\mathrm{pH}-\mathrm{p}K_a)}}$$

$$\phi_{ion} = 1 - \phi_n$$

Where ϕ_n is the neutral fraction and ϕ_{ion} is the ionised fraction. a = 1 for acids and -1 for bases.

4.15

$$\phi_n = \frac{1}{1 + 10^{\mathrm{pH}-\mathrm{p}K_{a_{acid}}} + 10^{\mathrm{p}K_{a_{base}}-\mathrm{pH}}}$$

$$\phi_- = \phi_n \cdot 10^{\mathrm{pH}-\mathrm{p}K_{a_{acid}}}$$

$$\phi_+ = \phi_n \cdot 10^{\mathrm{p}K_{a_{base}}-\mathrm{pH}}$$

Where ϕ_- is the anionic fraction and ϕ_+ is for the cationic fraction.

4.16

$$\log K_{OC} = \log\left(\phi_n \cdot 10^{0.54 \cdot \log P_n + 1.11} + \phi_{ion} \cdot 10^{0.11 \cdot \log P_n + 1.54}\right)$$

For acidic APIs. Where Log P_n is the Log K_{ow} of the neutral molecule.

4.17

$$\log K_{OC} = \log(\phi_n \cdot 10^{0.37 \cdot \log P_n + 1.70} + \phi_{ion} \cdot 10^{pK_a^{0.65} \cdot f^{0.14}})$$

For basic APIs. Where *f* is a diffusion limiting factor equal to Kow/Kow+1.

4.18

$$\log K_{OC} = \log(\phi_n \cdot 10^{0.50 \cdot \log P_n + 1.13} + \phi_{-} \cdot 10^{0.11 \cdot \log P_n + 1.54} + \phi_{+} \cdot 10^{pK_a^{0.65} \cdot f^{0.14}})$$

For amphoteric APIs.

4.5 Results

4.5.1 API stability in 10 mM $CaCl_2$

All APIs in 10 mM $CaCl_2$ all showed some decrease in concentration over 120 hours shaking (Figure 4.3). Naproxen showed the lowest decrease in concentration finishing at 6 % lost and propranolol the greatest at 28 % compared to the 0 hours spike. After 24 hours ofloxacin and naproxen show an 'increase' in concentration compared to 0 hours but this is small, maximum of 6 % for naproxen and 2 % for ofloxacin, and can be attributed to experimental or analytical error.

The decreases of APIs in the 10 mM $CaCl_2$ solution can be attributed to degradation (abiotic and biotic) or sorption to the test vessel. Biodegradation cannot be removed at this stage, as whilst the centrifuge tubes were sterile the solution was not. The sorption to the test vessel could have been measured by emptying the tubes and washing the vessels with an amount of an appropriate solution (OECD 2000). This was not done for these samples.

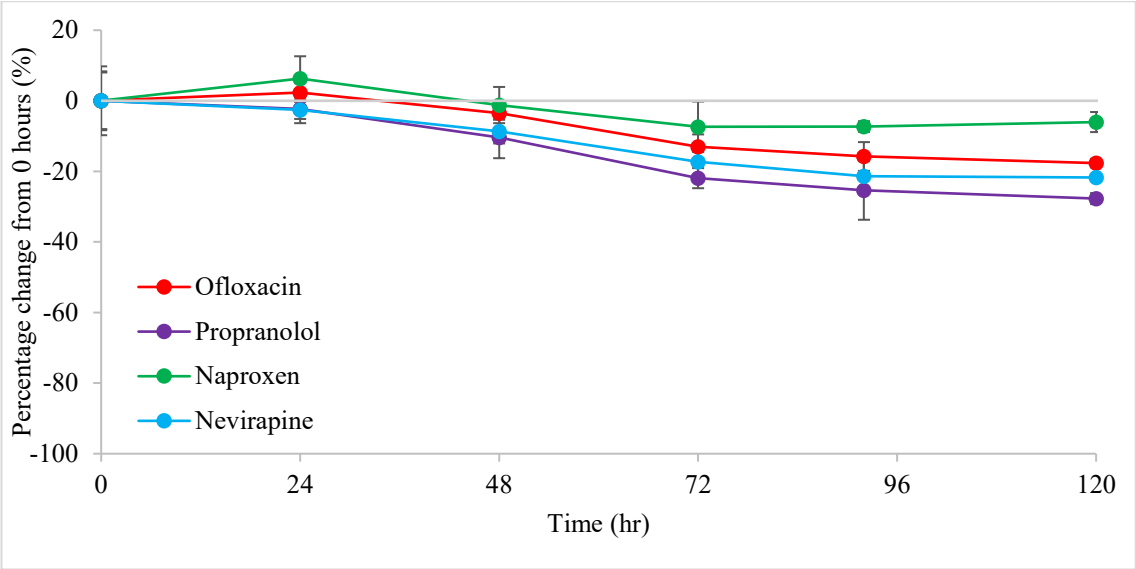


Figure 4.3 - Percentage decrease of APIs in 10 mM $CaCl_2$ solution compared to 0 hours (grey line) over 120 hours of shaking in the dark ($\bar{x}$ ± R.S.D. n = 3)

4.5.2 API loss from 10 mM $CaCl_2$ solution to soil

Ofloxacin loss from soil filtrates was characterised by very rapid loss from the initial spike of 500 µg L^{-1} to 14 and 12 µg L^{-1} in loam and sandy loam respectively (Figure 4.4). It reached equilibrium at 48 hours in loam and 24 hours in sandy loam (Table 4.6). At equilibrium the concentration of ofloxacin in the soil filtrates were very similar at 1.2 and 1.4 µg L^{-1} in loam and sandy loam respectively. Log K_{oc} was higher in the sandy loam soil (Table 4.7). The Franco and Trapp (2008) model data did not match the Log K_{oc} from the experiment and under-predicted the amount of sorption likely to take place (Table 4.7).

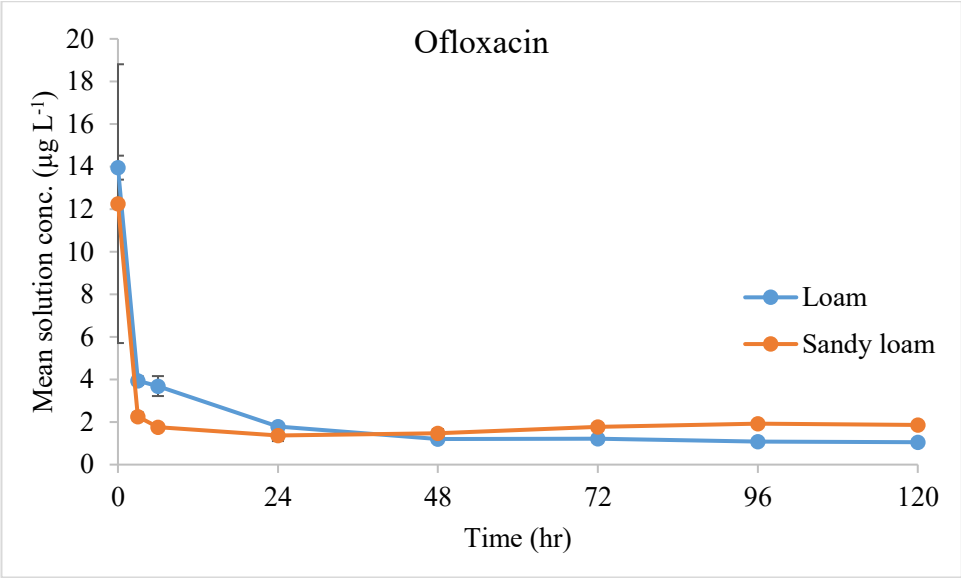


Figure 4.4 - Ofloxacin concentration in soil filtrates over time ($\bar{x}$ ± S.D. n = 4-6). Error bars are present for all data points but not visible in some cases.

Table 4.6 - Time required to reach sorption equilibrium* (hr)

	Loam	**Sandy loam**
Ofloxacin	48	24
Propranolol	72	48
Naproxen	48	24
Nevirapine	72	48

* Calculated using Equation 4.5

Table 4.7 – Sorption properties at equilibrium ($\bar{x}$ ± S.D. n = 4 or 6)

API	Soil	Conc. (µg L^{-1})	Loss from filtrate (%)	K_d (mL g^{-1})	Log K_{oc} (mL g OC^{-1})	Predicted* Log K_{oc} (mL g OC^{-1})
Ofl	L	1.2 ± 0.1	99.8 ± 0.0	2092.6 ± 277.6	5.0 ± 0.1	2.6
Prop	L	27.3 ± 1.2	86.4 ± 0.6	32.8 ± 1.6	3.2 ± 0.02	4.3
Nap	L	33.9 ± 2.0	19.1 ± 4.9	1.4 ± 0.2	1.8 ± 0.1	1.9
Nev	L	44.4 ± 1.9	15.7 ± 3.6	1.4 ± 0.3	1.9 ± 0.1	1.8
Ofl	SL	1.4 ± 0.3	99.7 ± 0.1	1875.5 ± 337.3	5.4 ± 0.1	
Prop	SL	107.4 ± 19.8	54.6 ± 0.6	6.0 ± 0.1	3.0 ± 0.03	
Nap	SL	38.3 ± 3.3	7.3 ± 6.8	0.4 ± 0.4	1.5 ± 0.7	2.1
Nev	SL	57.8 ± 7.0	0.9 ± 12.1	0.3 ± 0.1	1.6 ± 0.1	1.8

Ofl = ofloxacin, prop = propranolol, nap = naproxen and nev = nevirapine

*Predicted using Franco and Trapp (2008)

Propranolol was characterised by a rapid loss from the filtrate in both soils, before starting to plateau after 24 hours of shaking (Figure 4.5). Propranolol reached sorption equilibrium after 72 hours in loam and 48 hours in sandy loam (Table 4.6). Its initial loss from filtrate was greatest in the loam soil, where the added concentration reduced from 200 to 107 µg L^{-1} immediately after addition, compared with the sandy loam where there was no initial loss. The K_d and Log K_{oc} at equilibrium was higher in the loam than the sandy loam (Table 4.7). The Franco and Trapp model over-predicted the sorption of propranolol to soil, significantly increasing the Log K_{oc} compared to the experimental data (Table 4.7).

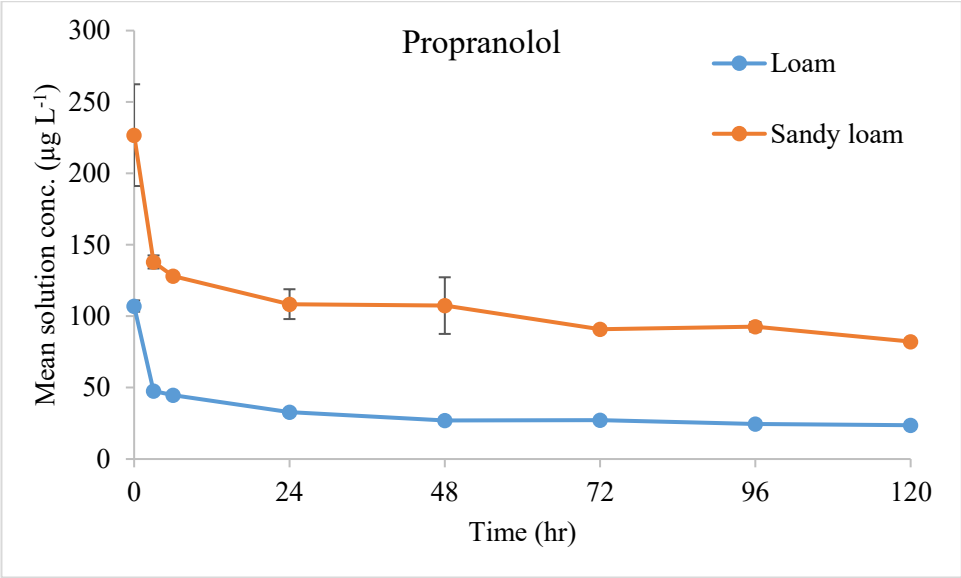


Figure 4.5 -Propranolol concentration in soil filtrate over time ($\bar{x}$ ± S.D. n = 4-6). Error bars are present for all data points but not visible in some cases.

Naproxen concentrations changed little after addition to the soil : $CaCl_2$ for the duration of the experiment (Figure 4.6). There was a small but significant decrease in filtrate concentration between the 0 hour samples and 120 hour samples for both soils (*t*-test two sample assuming equal variance, $p \leq 0.05$). In the loam soil filtrate, naproxen reached equilibrium at 48 hours and at 24 hours in the sandy loam (Table 4.6). The measured concentration at 0 hours in the sandy loam soil was greater than the nominal concentration of 30 µg L^{-1} due to variations in the spike solution (Table 4.7). As a result of this loss from filtrate, K_d and Log K_{oc} for both soils were calculated using the concentration measured immediately after addition as no loss from soil filtrate had occurred at this time (Table 4.7). K_d and Log K_{oc} were lower in the sandy loam soil.the Franco and Trapp (2008) model for acidic compounds allows the pH to be varied. In the case of the loam soil at the start pH of 7.2 (Figure 4.8) the model predicts the Log K_{oc} very well (Table 4.7). In the sandy loam the predicted Log K_{oc} is within the error of the experimental value.

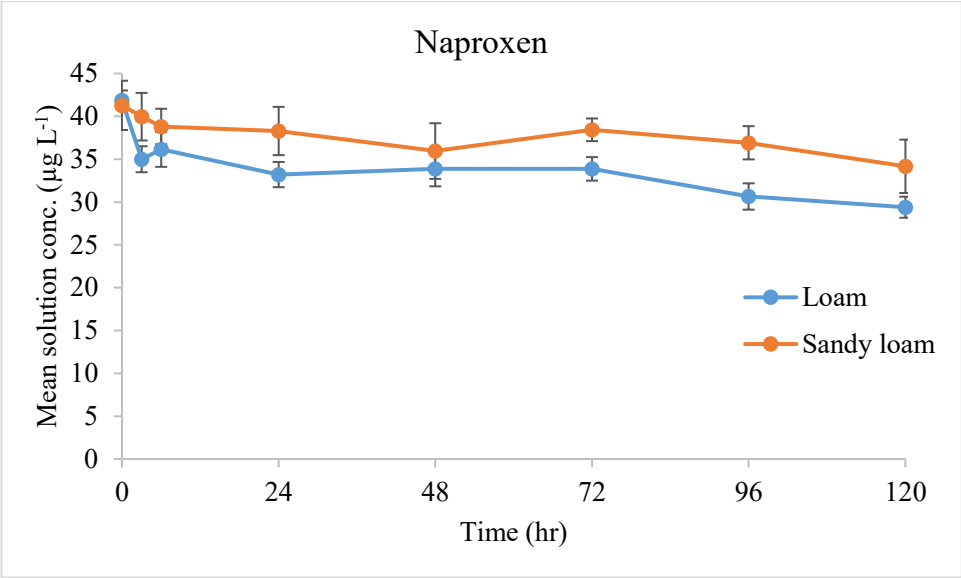


Figure 4.6 - Naproxen concentration in soil filtrate over time ($\bar{x}$ ± S.D. n = 4-6)

Nevirapine profiles were similar to naproxen with very little change over time (Figure 4.7). The concentrations in samples containing loam soil had decreased by 15.7 % at the equilibrium time of 72 hours (Table 4.6). As was observed with naproxen the added nevirapine concentration was higher than the nominal concentration so the concentration at 0 hours was used to calculate the sorption parameters. The K_d and Log K_{oc} were lower in the sandy loam samples (0.3 mL g^{-1} and 1.6 mL g OC^{-1} respectively) compared with loam (1.4 mL g^{-1} and 1.9 mL g OC^{-1} respectively) (Table 4.7). The Franco and Trapp (2008) model predicted the Log K_{oc} at the loam soil pH very well, whereas in the sand loam soil the predicted value was slight over-estimated Table 4.7.

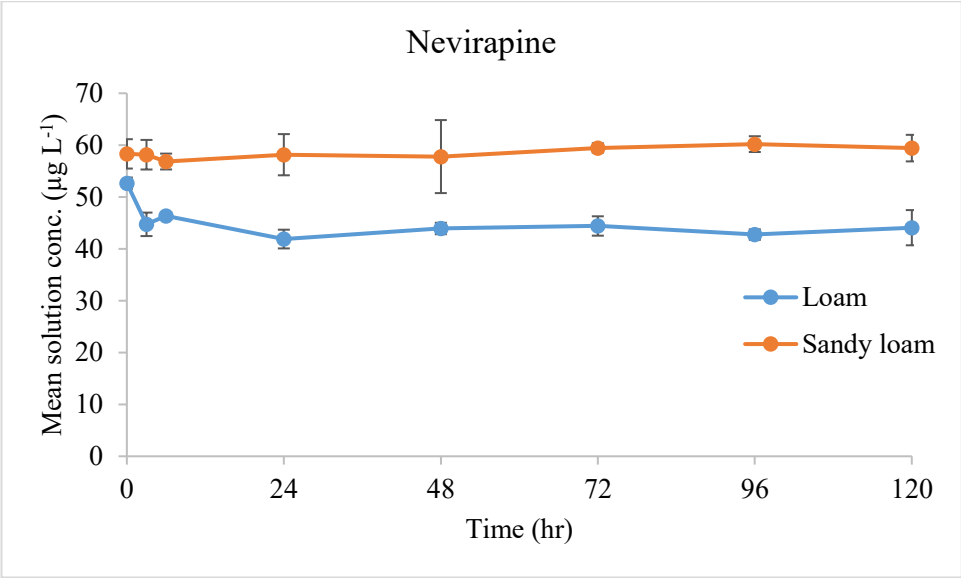


Figure 4.7 - Change in nevirapine concentration in soil filtrate over time ($\bar{x}$ ± S.D. n = 4-6)

4.5.2.1 Soil suspension changes

The pH and DOC concentrations of the soil suspensions showed that there was variation in matrix properties after a period of shaking. The initial pH of the loam soils was 7.2 and decreased to 6.7 over 120 hours (Figure 4.8). In contrast, the initial pH of the sandy loam soil was 6.1, increasing to 6.6 by the end of the experiment. The DOC concentration profile showed that levels were higher in the loam soil filtrate, throughout the experiments increasing from 30 mg L^{-1} to 44 mg L^{-1} (Figure 4.9), while that for sandy loam was 3.6 mg L^{-1}, increasing to 9.4 mg L^{-1}.

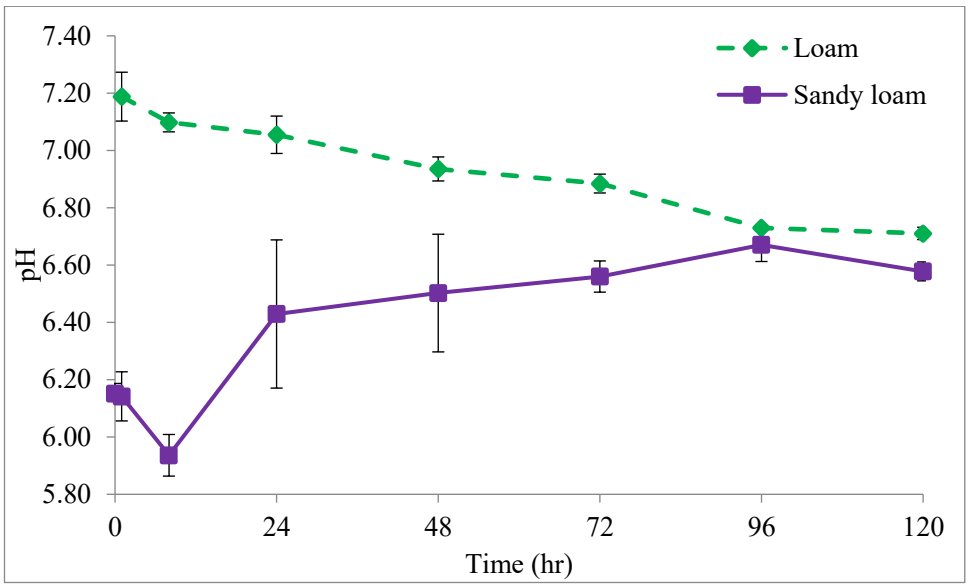


Figure 4.8 - pH of soil suspensions over 120 hours shaking ($\bar{x}$ ± S.D. n = 3)

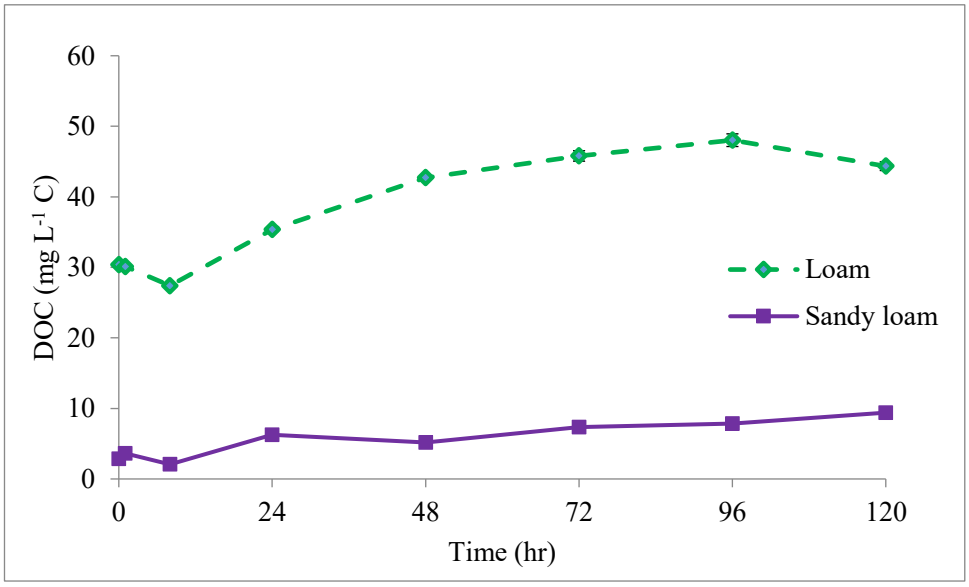


Figure 4.9 - DOC in soil filtrate over 120 hours shaking ($\bar{x}$ ± S.D. n = 3)

4.5.2.2 Overall charge on soils

The overall charge on soil was identified by comparing the pH measured in reverse osmosis (RO) water to that in 10 mM $CaCl_2$. Both soils were found to be negatively charged overall as the pH was higher in the RO water in both cases by approximately 0.5 pH units (Table 4.8) (Rowell 1994)

Table 4.8 - pH of soil in reverse osmosis water and 10 mM $CaCl_2$ ($\bar{x}$ ± S.D. n = 5)

	pH RO water	pH 10Mm $CaCl_2$	Overall charge
Loam	7.53 ± 0.05	7.11 ± 0.03	Negative
Sandy loam	6.67 ± 0.06	5.97 ± 0.05	Negative

4.5.3 Sorption isotherms

In most cases the APIs fitted the Freundlich isotherm best as the R^2 was greater compared with the Langmuir isotherm (Table 4.9). The only exception was propranolol in the loam soil samples, where the Langmuir isotherm had a slightly greater R^2, although the Freundlich isotherm also fitted well. K_F values are not comparable when 1/n values differ, as the calculation is dependent on 1/n, the slope of the isotherm (Martínez-Hernández et al. 2014 330). Where this occurred, K_d values were used to compare sorption affinity. The linearity of the Freundlich isotherms was indicated by the 1/n value; most APIs in both soils showed non-linear isotherms. Naproxen was linear in loam samples (1/n = 0.99), while nevirapine was close to linear (1/n = 10.94). Where 1/n < 1, this shows that as initial suspension concentration increases the adsorption decreases, indicating saturation of sorption sites.

In the case of Langmuir isotherms, those with a R^2 greater than 0.9 (ofloxacin and propranolol in both soils), ofloxacin in loam samples had the greatest concentration that the soil can retain, i.e. M is greatest. There was a wide range of M shown

(0.60 – 40.65 µg g^{-1}) which indicates that there was a difference in the adsorption affinity of the soils to the APIs which fit this isotherm. K_L has been related to potential bonding energy between the absorbant and the API but this relationship is unclear so it will not be discussed further here (EPA 1992).

Naproxen and nevirapine shown a great deal of variation in the case if the Langmuir isotherms error bars (Figure 4.11). This is the results of the measured concentration in the soil filtrate was slightly higher than the initial concentration in some cases due to analytical measurements and very little sorption. Once the isotherm had been calculated this expanded the error leading to large error bars when plotted.

Table 4.9 - Isotherm statistics of APIs in loam and sandy loam soils

		Freundlich			**Langmuir**		
	Soil	**K_F ($\mu g^{1-1/n} L^{1/n} g$)**	**1/n**	**R^2**	**K_L (L µg^{-1})**	**M (µg g^{-1})**	**R^2**
Ofloxacin	L	2.82	0.70	0.9992	0.06	40.65	0.9431
Propranolol	L	0.10	0.76	0.9775	0.14	1.07	0.9799
Naproxen	L	0.001	0.99	0.9736	-0.002	-0.73	0.0901
Nevirapine	L	0.002	0.94	0.9818	-0.003	-0.51	0.4385
Ofloxacin	SL	3.92	0.63	0.9918	0.11	35.84	0.9345
Propranolol	SL	0.04	0.65	0.9627	0.09	0.60	0.9595
Naproxen	SL	0.0006	0.59	0.9832	0.002	0.10	0.0075
Nevirapine	SL	0.0002	0.73	0.6145	-0.04	0.05	0.0392

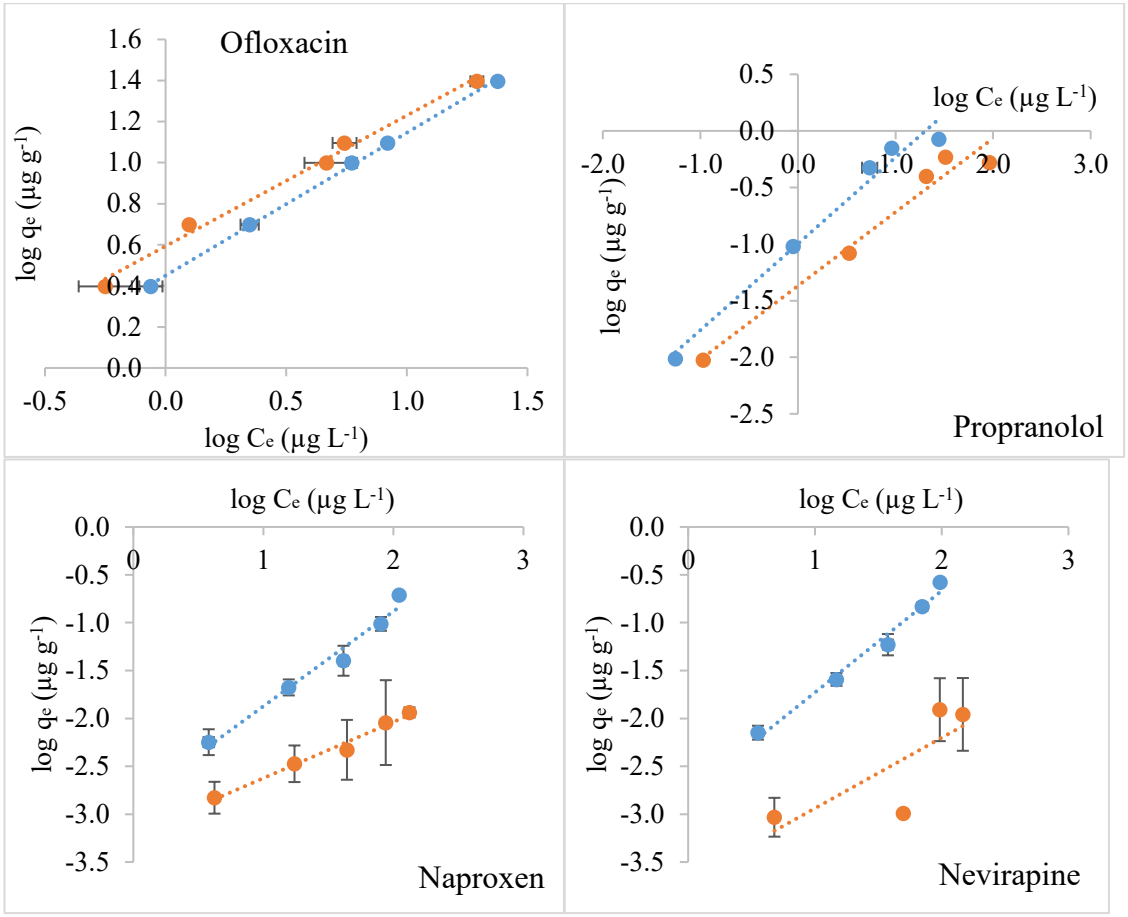


Figure 4.10 - Freundlich isotherms for all APIs in two soils ● = loam, ● = sandy loam. ($\bar{x}$ ± S.D. n = 4-9). Error bars are present for all x and y data but not visible in some cases.

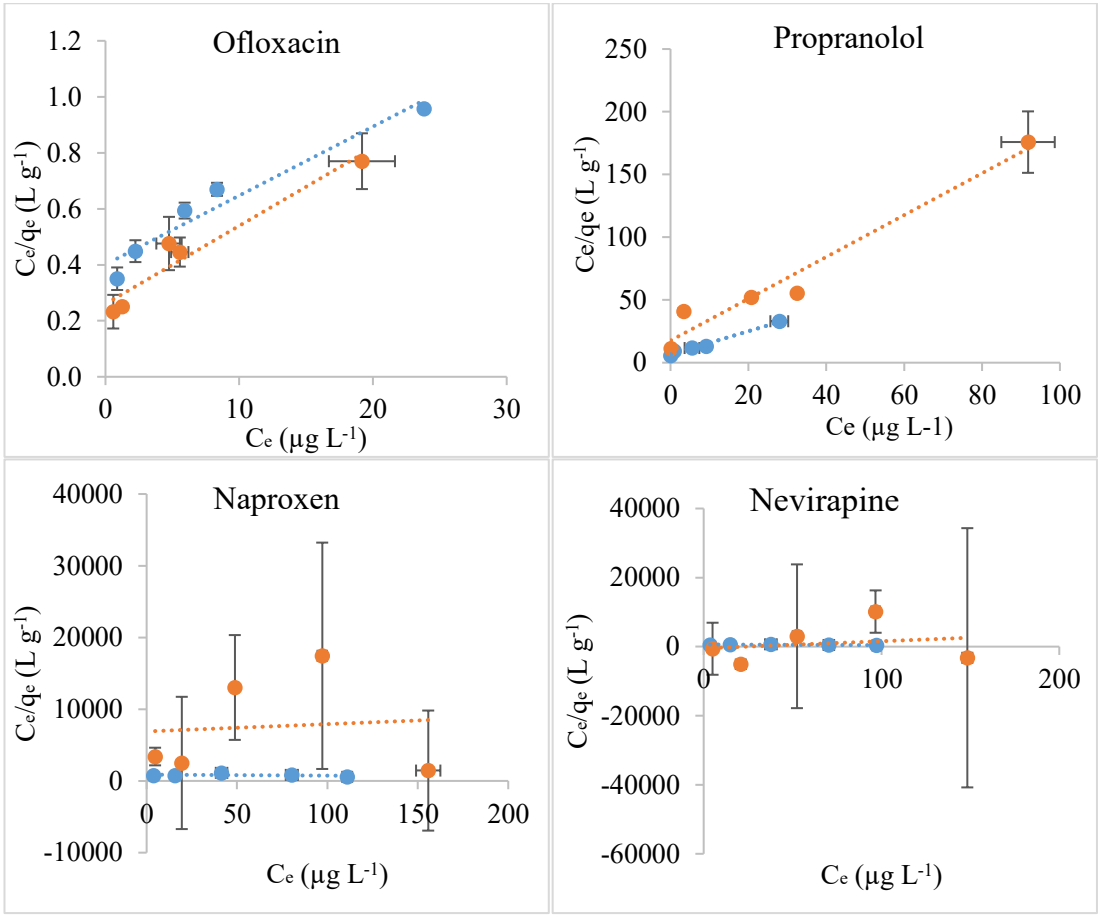


Figure 4.11 - Langmuir isotherms for all APIs in two soils ● = loam, ● = sandy loam. ($\bar{x}$ ± S.D. n = 4-9). Error bars are present for all x and y data but not visible in some cases.

4.5.4 Desorption

Ofloxacin desorption into fresh 10 mM $CaCl_2$ solution was minimal in both soils, being 0.18 and 0.14 % in loam and sandy loam, respectively, at equilibrium (Table 4.10). Desorption equilibrium was reached in loam at 24 hours and at 6 hours in sandy loam (Table 4.11). Ofloxacin sorption was largely irreversible in these soils indicated by the K_{des} values being significantly higher than the K_d values (Table 4.7) (*t*-test two sample assuming equal variance, $p \leq 0.05$).

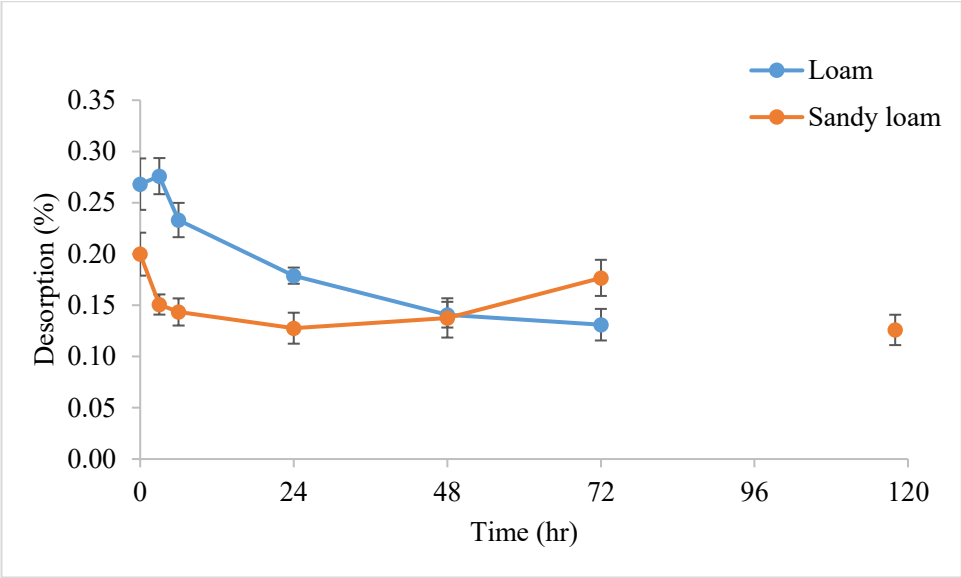


Figure 4.12 - Ofloxacin desorption ($\bar{x}$ ± S.D. n = 4 or 6). Gaps in line indicate where concentration went below LOQ

Table 4.10 - Desorption statistics ($\bar{x}$ ± S.D. n = 4 or 6)

	Soil	Concentration adsorbed (µg L^{-1})	Filtrate concentration (µg L^{-1})	Desorption (%)	K_{des} (mL g^{-1})
Ofloxacin	L	499.27	0.98 ± 0.04	0.18 ± 0.01	2795.21 ± 123.54
Propranolol	L	184.02	14.07 ± 1.16	6.57 ± 0.65	71.72 ± 8.04
Naproxen	L	9.87	6.96 ± 0.79	47.05 ± 6.84	5.83 ± 1.66
Nevirapine	L	27.66	12.81 ± 0.94	34.09 ± 2.70	9.74 ± 1.15
Ofloxacin	SL	499.18	0.78 ± 0.08	0.14 ± 0.01	3506.36 ± 33.89
Propranolol	SL	122.48	41.20 ± 2.42	27.63 ± 2.11	13.18 ± 1.35
Naproxen	SL	4.50	4.16 ± 0.26	46.20 ± 5.55	5.95 ± 1.32
Nevirapine	SL	11.31	6.19 ± 0.10	21.73 ± 0.86	18.04 ± 0.01

Table 4.11 – Time taken to reach desorption equilibrium (hr)

	Loam	Sandy loam
Ofloxacin	24	6
Propranolol	24	96
Naproxen	48	24
Nevirapine	48	24

The propranolol desorption rate was highest from 0-3 hours and then decreased. Sandy loam showed the greatest desorption of the two soils with 27.63 % desorbed compared to 6.57 % in the loam (Table 4.10). The time to equilibrium varied between the soils with loam taking 24 hours and sandy loam taking 96 hours. The majority of propranolol adsorbed was not removed from these soils, indicated by the K_{des} values being significantly higher than the K_d values (Table 4.7) (*t*-test two sample assuming equal variance, $p \leq 0.05$).

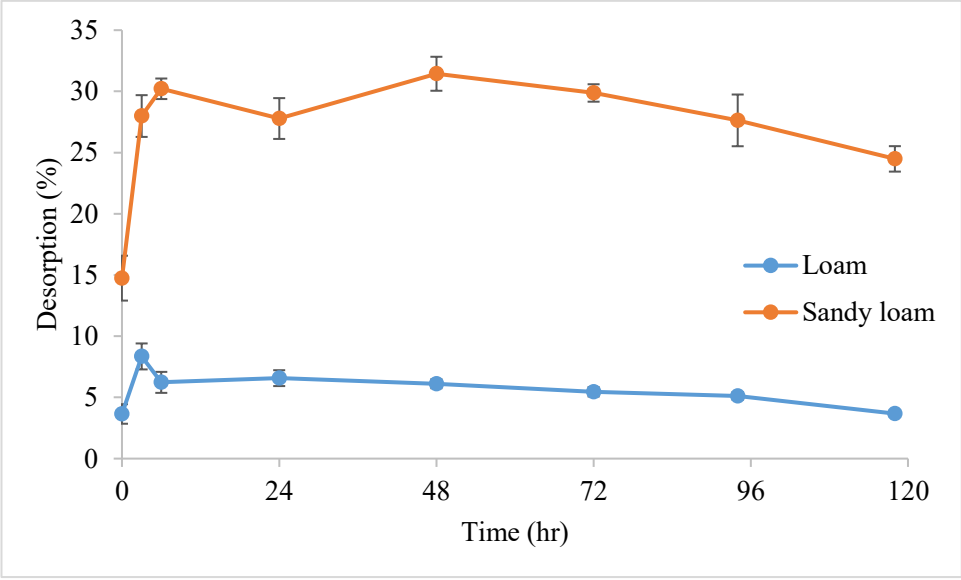


Figure 4.13 - Propranolol desorption ($\bar{x}$ ± S.D. n = 6). Error bars are present for all data points but not visible in some cases.

Naproxen desorption was characterised by a similar rapid loss from both soils which accounted for 47.05 and 46.20 % of the sorbed API concentration for loam and

sandy loam respectively (Figure 4.14). The different sorbed concentrations allowed for different concentrations of the API to be desorbed (Table 4.10). Naproxen sorption showed some reversibility (approximately 50 %) in these soils indicated by the K_{des} values being significantly higher than the K_d values but to a smaller extent that ofloxacin and propranolol (Table 4.7) (*t*-test two sample assuming equal variance, $p \leq 0.05$).

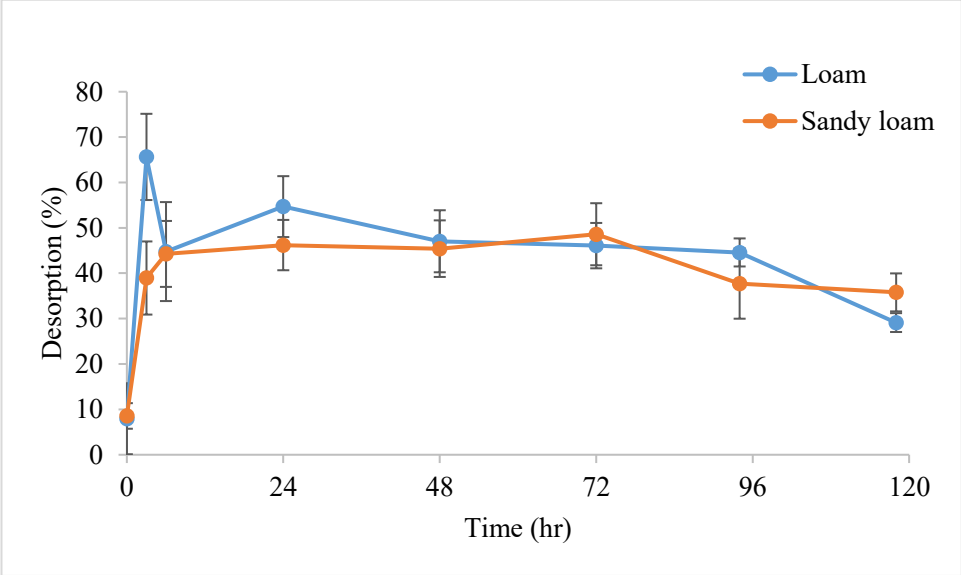


Figure 4.14- Naproxen desorption ($\bar{x}$ ± S.D. n = 6)

For nevirapine, most desorption in loam samples occurred within 3 hours, before a small decrease in the desorbed concentration then reaching although equilibrium did not occur before 48 hours (Figure 4.15). Sandy loam showed a smoother loss from soil before equilibrating at 24 hours (Figure 4.15). The greatest desorption was measured in loam at 34.09 % compared to 21.73 % in sandy loam. Nevirapine sorption showed some reversibility (34 % for loam and 22 % for sandy loam) in these soils indicated by the K_{des} values being higher than the K_d values but the difference is not as big as ofloxacin and propranolol (Table 4.7) (*t*-test two sample assuming equal variance, $p \leq 0.05$).

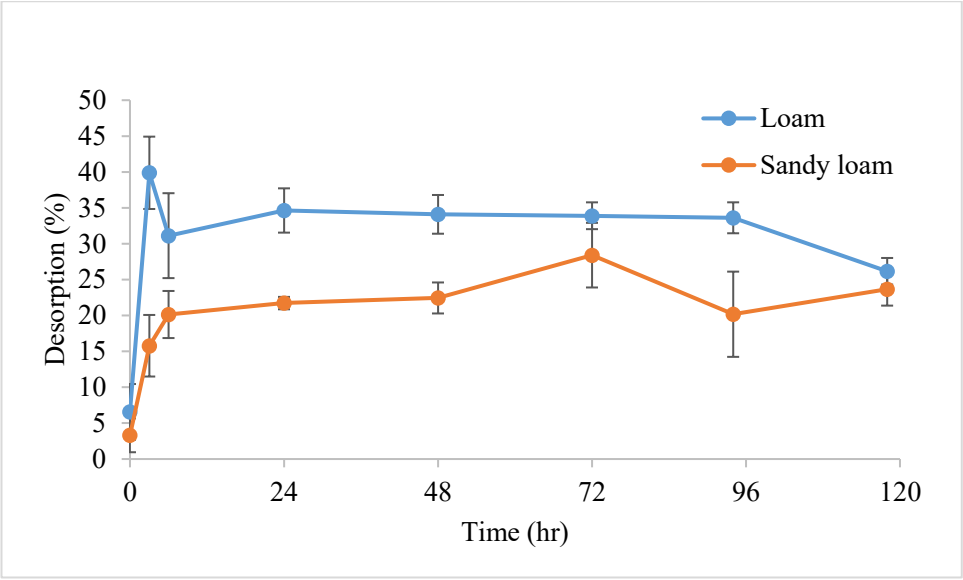


Figure 4.15 - Nevirapine desorption ($\bar{x}$ ± S.D. n = 6)

4.5.4.1 Physico-chemical parameters

pH and DOC concentrations varied after replacing the 10 mM $CaCl_2$ solution with a fresh aliquot. The loam soil pH decreased from 6.57 to 5.97 immediately after replacing the solution, while that for sandy loam was unchanged (Figure 4.16).

$CaCl_2$ replacement reduced the DOC leached into the fresh 10 mM $CaCl_2$ compared to the concentrations in the replaced solution (Figure 4.17). The loam soil demonstrated a large decrease after replacement from 41.66 to 6.91 mg L^{-1} and the sandy loam DOC concentration decreased from 8.00 to 1.30 mg L^{-1}. The loam DOC gradually increases over time from 6.91 to 10.02 mg L^{-1} and sandy loam had a smaller increase from 1.30 to 3.50 mg L^{-1} both of these are statistically significant (*t*-test two sample assuming equal variance, $p \leq 0.05$). Calculating the partition coefficient (K_d) for the DOC over the 120 hour shaking period normalises the concentration for the concentration of organic carbon in the soil at each time point, having taken into account DOC liberated from soil and removed from the sorption experiment (Figure 4.18). Initially due to the low DOC solution concentration of the sandy loam this has a high K_d but this decreases

over time until 48 hours where this levels off closer to the loam soil but they are significantly different (*t*-test two sample assuming equal variance, $p \leq 0.05$).

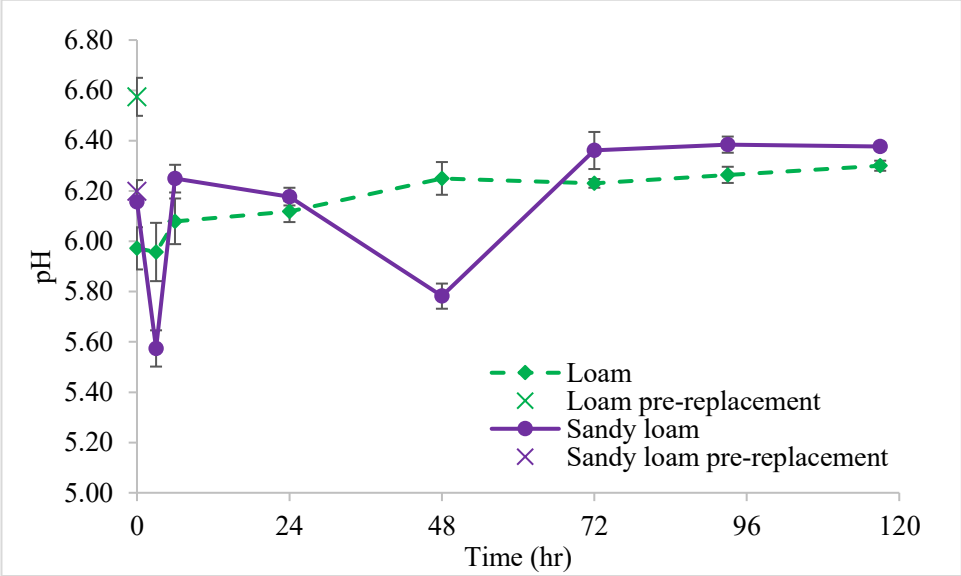


Figure 4.16 - pH after 10 mM $CaCl_2$ replacement ($\bar{x}$ ± S.D. n = 3)

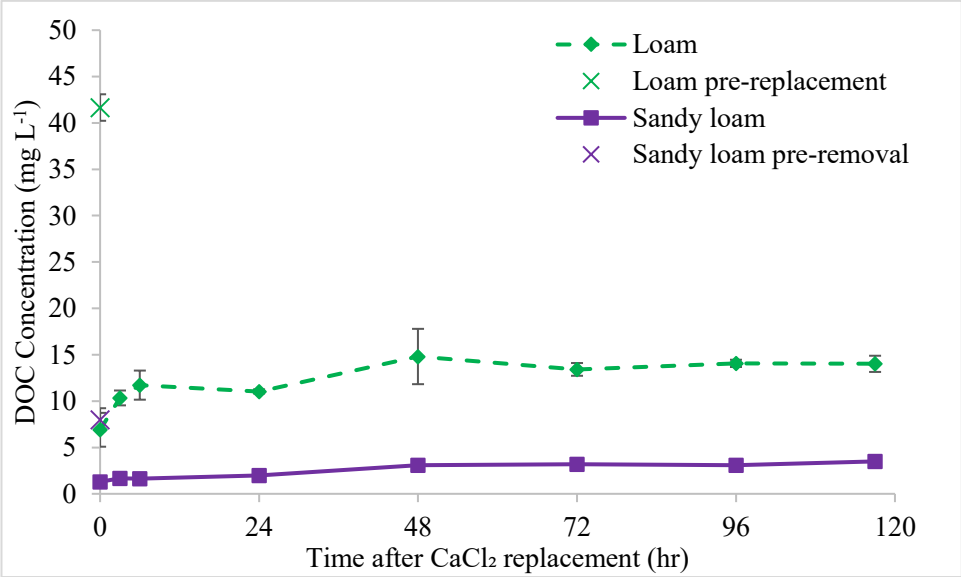


Figure 4.17 - DOC concentration after 10 mM $CaCl_2$ replacement ($\bar{x}$ ± S.D. n = 9)

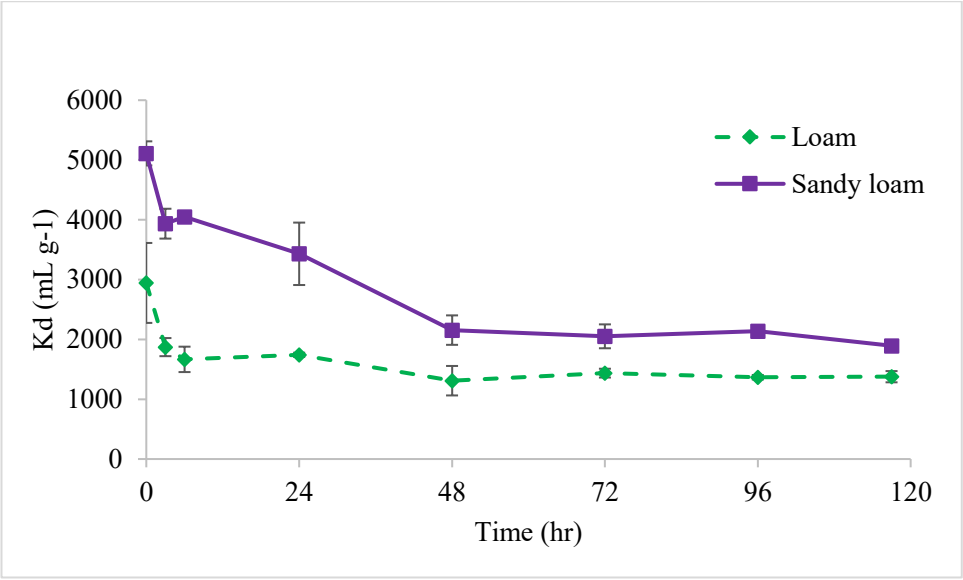


Figure 4.18 - Partition coefficient (K_d) of DOC in soil solutions after 10 mM $CaCl_2$ replacement ($\bar{x} \pm$ S.D. n = 9). Error bars are present for all data points but not visible in some cases.

4.6 Discussion

The data from these experiments showed large variation in the sorption behaviour of the four APIs. The data was considered with respect to the sorption-desorption behaviour of each API, the soil properties and changes in soil properties during the experiment.

4.6.1 Stability of APIs in 10 mM $CaCl_2$

The stability of the APIs in 10 mM $CaCl_2$ showed that there was the tendency for all APIs concentrations to decrease over 120 hours of shaking to varying extents. Propranolol showed the greatest decrease from the initial concentration over time where after 120 hours of shaking 73 % of the initial spike remained in solution. Nevirapine had 79 %, ofloxacin 82 % and naproxen 94 % of the initial spike remained in solution after 120 hours shaking. However, for all the APIs at the time it took to reach equilibrium in soils (Table 4.6) all of the APIs were ≥ 80 % of the initial spike in the control solution.

The reasons behind this loss of APIs may be due to sorption to the vessel wall or degradation of the APIs via biotic or abiotic pathways (OECD 2000). Whilst this study was conducted in sterile centrifuge tubes, the 10 mM $CaCl_2$ solution was not abiotic. Data on the loss of APIs to the surface of the container cannot be extrapolated from the control solution to the soil solutions as the presence of soil greatly reduces the loss to the tubes due to the increased availability of sorption sites (OECD 2000). The soil will also add more microorganisms that will impact the biotic degradation of the APIs and change the pH of the experimental matrix (Vasudevan et al. 2009; Qin et al. 2015). For these reasons, the sorption data was not corrected to account for loss from10 mM $CaCl_2$ solutions as it is not representative of the test systems.

4.6.2 Ofloxacin

Ofloxacin concentrations were characterised by rapid and almost complete adsorption to both soils (Figure 4.4). This API has two pK_a values, for the carboxylic acid (5.97) and the piperizinyl ring (8.28). The pH of the soils varied during the experiment, from 6.7 - 7.2 (loam) and 6.1 - 6.6 (sandy loam) (Figure 4.8). For the sandy loam soil the carboxylic group of ofloxacin would be 60 - 80 % ionised (-ve charge), while the amino group would be almost completely ionised (99-98 % +ve charge). For the loam soil, the carboxylic group would be 94 - 85 % ionised and the piperizinyl ring would be 93 - 97 % ionised (Figure 4.1). This effectively means that at the range of soil pH measured in each incubation, ofloxacin is a zwitterion. These charged groups would enable ion-ion and ion-diople interactions, affecting the sorption to mineral clay surfaces as well as soil organic matter. The neutral molecules of ofloxacin in the loam soil (10 %) could have been adsorbed by hydrophobic interactions, hydrogen bonding, ion–dipole, and aromatic electron donor–acceptor processes (Conkle et al. 2010).

To identify greater differences in sorption between these two soils a different soil: solution ratio would be necessary. According to the OCED 106 guideline this could be changed to a minimum soil weight of at least 1 g of soil, and a ratio of 1:100 (OECD 2000). This would allow for larger solution concentrations to be analysed with greater accuracy, it may also allow for a lower spike concentration to be used that is more in line with environmental concentrations.

The high potential for ofloxacin to sorb to both soils is demonstrated by the large Log K_{oc} at equilibrium (Table 4.7). The different Log K_{oc} values show that organic carbon is not the only driving force for loss of ofloxacin from soil suspensions (5.00 and 5.44 in loam and sandy loam, respectively). There is evidence that the inorganic constituents of soils exert greatest control over ofloxacin sorption (Pan et al. 2012; Zhou et al. 2014). This is possibly due to the negatively-charged carboxylic group complexing with metal cations such as calcium, aluminium and iron in the soil (Gu et al. 2005; Zhang et al. 2007). There may be competition between soil organic matter and ofloxacin for sorption sites on mineral particle surfaces (Zhou et al. 2014), which could be why the soil with higher organic carbon content (loam) had a lower Log K_{oc} than the sandy loam soil in this experiment. The many different types of interactions with soils this zwitterion can achieve may explain why desorption was largely irreversible in both soils with the K_{des} being significantly higher than K_d (Table 4.7 and Table 4.10).

The predicted sorption of ofloxacin was largely underestimated by the Franco and Trapp (2008) model (Table 4.12). The low Log K_{oc} predicted by this model suggests that ofloxacin would not be persistent in the soil compartment and would potentially pose a threat to groundwater, as it is not predicted to be strongly sorbed to soils (ECETOC 2013a). If this value was used for risk assessment then attention may be diverted from looking at risks to the soil compartment onto risk in groundwater leading to unknown risks being posed to soil flora and fauna. Modelling should go hand in hand with

experimental data until a model has been developed that can accurately predict the fate of zwitterions in soil environments. This model has limitations that are due to the standardising of K_d to the organic content of the soils as discussed in Chapter 1. The sorption to clay and other inorganic surfaces in soils is important for controlling the fate of zwitterionic APIs (Pan et al. 2012). There appears to be a lack of examples using this model for estimating the sorption of zwitterions in soils. Further development of models and use of models in this circumstances would aid understanding on the factors controlling the fate of zwitterions in soils.

Sorption isotherms in both soils had a 1/n value less than 1 indicating a saturation of sorption sites at higher concentrations, as relative sorption decreased as concentration increased. The sorption capacities (K_F) were not comparable due to the differences in 1/n but K_d values showed that ofloxacin had the greatest affinity for loam and was the most sorbed API of those studied (Table 4.7). Published isotherms vary extensively and are dependent on soil characteristics (Table 4.12). Ofloxacin isotherms reported for most soils tend to be non-linear, reaching saturation.

Table 4.12 - Ofloxacin Freundlich isotherm data from literature

Soil	K_d	Log K_{oc}	K_F	1/n	R^2	Ref.
pH 7.1, OC 1.98%, CEC 30.6 $cmol^{-1}$ kg	2093	5.0	2.8	1/n = 1.44	0.995	This study
pH 6.0, OC 0.67 %, CEC 7.3 $cmol^{-1}$ kg	1876	5.44	4.0	1/n = 0.63	0.976	This study
		2.61				Estimated using Franco and Trapp (2008)
pH 7.6, OM 10.4 g kg^{-1}, CEC 6.4 cmol kg^{-1}			13.2	n = 1.604	0.975	(Peng et al. 2014)
pH 3.5, OM 42.7 g kg^{-1}, CEC 7.4 cmol kg^{-1}			3260	n = 0.316	0.994	(Peng et al. 2014)
pH 4.3, OM 13.4 g kg^{-1}, CEC 25.7 cmol kg^{-1}			6730	n = 0.262	0.961	(Peng et al. 2014)
pH 4.3, OC 7.1 %	3554	5.7	3224	1/n = 0.94	0.991	(Drillia et al. 2005)
pH 6.8, OC 0.37 %	4087	5.5	832	1/n = 0.61	0.994	(Drillia et al. 2005)
pH 6.5, CEC 2.1 cmol kg^{-1}	75.8		1778	n = 0.46	0.992	(Zhou et al. 2014)
pH 7.0, CEC 8.2 cmol kg^{-1}	234		2041	n = 0.627	0.955	(Zhou et al. 2014)

4.6.3 Propranolol

Propranolol has a pK_a of 9.3 so over 99 % was in cationic form at the pH of both soils (Figure 4.1). As both soils had overall negative charges (Table 4.8) it was expected to adsorb strongly to these soils via electrostatic attraction (ter Laak et al. 2006b). This was reflected in the data, as there was rapid loss of the API from filtrate over 6 hours (Figure 4.5). Due to the positive

charge on the molecule at the measured pH of the soil suspension, the negatively-charged organic matter and clay is most likely the main control on adsorption (Franco et al. 2009; Martínez-Hernández et al. 2014).

The difference in Log K_{oc} between the two soils (Table 4.7) indicates that the organic carbon may not be the only soil parameter that drives loss of APIs from suspension. The loam soil had the highest Log K_{oc} for propranolol; this soil has a greater clay content (25.8 % compared to 6.5 % in sandy loam). Clay generally has a cation exchange capacity (CEC) at pH values found in soil systems because of isomorphous substitution (i.e. the replacement in the mineral structure of one cation with another of a different charge) and de-protonation of surface hydroxyl groups (the extent being dependent on pore water pH) while the overall magnitude of the CEC value depends on clay-type (Lees et al. 2016). CEC in lower and lower middle income countries that regularly use wastewater for irrigation has been mesured at 22 – 55 $cmol^{-1}$ kg (FAO/IIASA/ISRIC/ISS-CAS/JRC 2009). The higher the CEC value the more sorption sites are potentially available (Lees et al. 2016), reflecting the CECs of the two soils used in this study; the loam soil had a CEC at 33 $cmol^{-1}$ kg compared to the sandy loam soil at 7.5 $cmol^{-1}$ kg. This may explain the differences in the Log K_{oc} values (Table 4.13).

Sorption of propranolol was over-estimated when using the Franco and Trapp (2008) model compared to the experiment data from this study and relevent litearture studies (Table 4.13). This model has been shown in the literature to over-estimate basic compounds in soil solutions (Al-Khazrajy et al. 2016). The failure of the model to predict Log K_{oc} for propranolol and ofloxacin shows that this problem is complex due to the range of interactions that can occur between these APIs and soil particles (Al-Khazrajy et al. 2016). Improved models would increase the chance that these API-soil interactions could be predicted accurately. Better models that are mechanistic and process-based that consider relevent boundary conditions would aid the risk assessment procedure and reduce costly laboratory work required (Schaffer et al. 2012).

The results from the isotherm experiments showed that propranolol fitted both the Freundlich and Langmuir isotherms ($R^2 > 0.94$). The Freundlich isotherms showed that both soils had non-linear isotherms and that as concentration increased relative sorption

decreased due to the saturation of sorption sites (1/n <1) (Table 4.9). K_F was not comparable due to the differences in the 1/n value. From the K_d values propranolol had a greater sorption affinity in the loam soil. Literature values for the Freundlich isotherms also show this variation depending on soil characteristics (Table 4.13). The Langmuir isotherm indicated that the loam soil had the greatest capacity for sorption of propranolol (M was greatest) which was in agreement with the K_d values.

Table 4.13 – Published propranolol Freundlich isotherm data

Soil	K_d	Log K_{oc}	K_F	1/n	R^2	Ref.
pH 7.1, OC 1.98%, CEC 30.6 $cmol^{-1}$ kg	33	3.2	10	1.44	0.995	This study
pH 6.0, OC 0.67 %, CEC 7.3 $cmol^{-1}$ kg	6	3.0	23	0.65	0.963	This study
		4.27				Estimated using Franco and Trapp (2008)
pH 4.3, OC 7.1 %	199	3.5	207	0.85	0.996	(Drillia et al. 2005)
pH 6.8, OC 0.37 %	16	3.6	7.15	1.43	0.994	(Drillia et al. 2005)
pH 5.62, OC 2.1 %, CEC 6.2 cmol kg^{-1}	47	3.3	209	0.93	0.999	(Maszkowska et al. 2014)
pH 6.22, OC 14.9 %, CEC 53.1 cmol kg^{-1}	154	3.0	62	1.02	0.985	(Maszkowska et al. 2014)
pH 6.65, OC 19.4 %, CEC 85.6 cmol kg^{-1}	161	2.9	268	0.85	0.999	(Maszkowska et al. 2014)

Desorption of propranolol from the soils was characterised by a large difference of percentage desorption between the two soils, although the overall pattern of desorption

was similar (Figure 4.13). Sandy loam samples desorbed the API to a greater extent, showing more weaker sorption than in loam as the difference between K_d and K_{des} was smaller (Zhang et al. 2017). This indicates that propranolol sorbed to a similar soil would be more mobile in soil environments and potentially impact groundwater through leaching compared with ofloxacin, which is strongly sorbed. Similar results have been found for other cationic APIs, such as atenolol (Martínez-Hernández et al. 2014; Maszkowska et al. 2014). Soils with a lower organic carbon content tend to have more reversible sorption, which was observed in these experiments (Maszkowska et al. 2014).

4.6.4 Naproxen

Naproxen (pK_a 4.2) would have been predominantly anionic (-ve charged) in both soils, reflected in the lower adsorption observed due to charge repulsion by negatively-charged soil surface sites. However, sorption did occur (19.1 % for loam and 7.3 % for sandy loam) and may be due to the affinity of aromatic groups on the molecule for the organic content of the soils (Chefetz et al. 2008; Lees et al. 2016).

A large range of K_d values have been reported for naproxen (Table 4.14), due to the nature and degree of hydrophobicity of the natural organic matter (Chefetz et al. 2008; Xu et al. 2009b; Durán-Álvarez et al. 2012; Martínez-Hernández et al. 2014). The small difference in the loss of the API in the soil filtrates between soil types may be evidence for the lack of sorption being less influenced by charge repulsion from charged sites on soil particles and more influenced by sorption to other soil components which are not dependent on ionic charge. The aromatic rings on the API can facilitate π-π interactions with aromatic moeities of the soil organic matter, counteracting the charge repulsion of the clay minerals and is influenced by the nature of the natural organic matter, although the same is true for propranolol (Chefetz et al. 2008; Martínez-Hernández et al. 2014). The organic matter probably plays more of a role in the π-π interactions with more mature

and humified soil organic matter promoting sorption of naproxen due to relatively greater amounts of aromatic and alkyl moieties (Chefetz et al. 2000; Chefetz et al. 2008). However, naproxen is less hydrophillic than propranolol and so less likely to interact with the inorganic surfaces sites in the soil (Martínez-Hernández et al. 2014). As naproxen does not have a strong sorption affinity for soils it has low Log K_{oc} values (Table 4.14). Correcting the K_d for the fraction of organic carbon present in the soils will lead to a equal Log K_{oc}; as this was not the case for naproxen in the loam and sandy loam, it would appear that the the inorganic surfaces must provide some sorption sites for naproxen (Chefetz et al. 2008).

Table 4.14 – Published naproxen K_d and Log K_{oc}

K_d	Log K_{oc}	Soil	Reference
1.4	1.84	pH 7.1, OC 1.98%, CEC 30.6 $cmol^{-1}$ kg	This study
0.4	1.50	pH 6.0, OC 0.67 %, CEC 7.3 $cmol^{-1}$ kg	This study
	2.07	pH 5.9	Estimated using Franco and Trapp (2008)
	1.90	pH 7.2	Estimated using Franco and Trapp (2008)
11	2.48	pH 6.3, TOC 3.77 %, 20 % clay	(Barron et al. 2009)
1.24	2.45	pH 7.54, OM 0.58 %, FOC 0.44 %, 3.6 % clay	(Xu et al. 2009b)
16.49	2.72	pH 7.14, OM 5.45 %, FOC 3.16 %, 18.1 % clay	(Xu et al. 2009b)
2.39	1.98	pH 8.01, TOC 25 mg g^{-1}, clay 45 %	(Durán-Álvarez et al. 2012)

The Franco and Trapp (2008) model predicted Log K_{oc} for soils of the same pH as the loam and sandy loam accurately within the experimental error (Table 4.7 and Table 4.14). This model has been successfully used in literature to predcit Log K_{oc} of naproxen and phenobarbital (another acidic compound) to natural sandy aquifer sediments at different pH (Schaffer et al. 2012). Although Al-Khazrajy (2016) found that this model underpredicted the sorption of acidic compounds to sediments, this may be due to the fact that this model was developed for soil so may not be directly transferrable to sediments {Boxall, 2012 #574}. The variations in the quality of Log K_{oc} predictions for this model reflects the complex nature of soils and sediments suggesting that a model with increased complexity is required that will include as many variables as possible such as clay content, associated CEC, pH and other factors discussed in Chapter 1.

Biodegradation has been reported as causing loss of naproxen from soil suspensions, through comparison of non-irradiated and irradiated soil samples (Durán-Alvarez et al. 2009; Monteiro et al. 2009; Lin et al. 2011). So loss from solution in this experiment cannot be attributed to sorption alone.

The Freundlich isotherm for both soils was a good fit (R^2 = >0.97). Neither soils fitted the Langmuir isotherm (R^2 = <0.09) (Table 4.9, Figure 4.10 and Figure 4.11). The loam Frendlich isotherm showed a linear regression (1/n = 0.99) but with a very low K_F (0.001), showing that saturation of sorption sites did not occur at the concentrations used (Table 4.9). The sandy loam Freundlich isotherm showed a non-linear regression (1/n = 0.59) indicating that as concentration increased relative sorption decreased due to the saturation of sorption sites. The low sorption of naproxen to soils which is shown by the low K_d values measured (1.4 mL g^{-1} in loam and 0.4 mL g^{-1} in sandy loam) is more likely to have been respobnsible. Similar low sorption rates have been reported, though higher K_F values (Table 4.15). The range of linearity for naproxen (and all APIs) in soils is due to the large variation in soil properties that have an impact on sorption. It is unclear from

considering reported sorption values, which soil parameter has most influence on sorption isotherm characteristics as there is significant variation (Table 4.15).

Table 4.15 – Published naproxen Freundlich isotherm data

Soil	K_d	K_F	n	R^2	Ref.
pH 7.1, OC 1.98%, CEC 30.6 $cmol^{-1}$ kg	1.4	0.001	1/n = 0.99	0.92	This study
pH 6.0, OC 0.67, CEC 7.3 $cmol^{-1}$ kg	0.4	0.001	1/n = 0.57	0.50	This study
pH 6.1, OM 38 g kg^{-1}, CEC 20.5 cmol kg^{-1}	6.5	16.2	1.4	1.00	(Zhang et al. 2017)
F_{oc} 4.6 %	356	764	0.629	0.95	(Vulava et al. 2016)
F_{oc} 1 %	3.4	9.8	0.827	0.95	(Vulava et al. 2016)
F_{oc} 9 %	58.7	78.5	0.944	0.97	(Vulava et al. 2016)

From the small concentration of naproxen that sorbed to the soil, almost half was desorbed (47 % in loam and 46 % in sandy loam) (Table 4.10). This was the greatest percent desorption from all APIs and indicates that an amount of the sorbed naproxen is readily reversible, as has been observed in other studies (Zhang et al. 2017). The desorption of naproxen shows that its movement through soil profiles may be retarded by sorption/desorption interactions but it will be more mobile and likely to permeate soil and transfer to surface and ground waters, than ofloxacin and propranolol. As K_{des} was greater than K_d for naproxen in both soils the sorption of naproxen can be classified as partially irreversible during the time of this experiment (Zhang et al. 2017). Other studies have reported that organic chemicals can take significantly longer (potentially months) to reach complete sorption or desorption equilibrium rather than the operational equilibrium used

in this study, so certain APIs may eventually be released from soil particles into the liquid phase, depending on degradation rates (Pignatello et al. 1996).

4.6.5 Nevirapine

At the experimental pH range measured during these experiments nevirapine was completely unionised (Figure 4.1). This resulted in minimal sorption during the experiment, constituting the lowest percentage loss from the filtrate of all the APIs, in both soils. The small amount of sorption that occurred in the soils was probably caused by hydrophobic interaction with organic material in soil, such as the humic and fulvic components (Schwarzenbach et al. 1993). This interaction was demonstrated by greater sorption in loam soil (15.7 %), which contained most organic carbon. As nevirapine has not been previously studied in soils, comparison is only possible with other neutral APIs. Similarly low sorption has been measured for carbamazepine (0.4 – 1.3 L kg^{-1}) and acetaminophen (0.5 L kg^{-1}) (Löffler et al. 2005; Martínez-Hernández et al. 2014) in soils with low K_d. Freundlich sorption isotherms for nevirapine only fitted in the loam soil (R^2 = 0.9522), where 1/n was close to 1 and the sorption could be described as linear and did not reach maximum sorption capacity (Table 4.9). Percentage desorption was highest in the loam soil (34.09 %) (Table 4.10) showing that the hydrophobic interactions with the organic material were not strong (Schwarzenbach et al. 1993). K_{des} was highest in the sandy loam soil showing that the small amount of sorption that occurred was less reversible than in the loam soil (Table 4.10).

As with naproxen, the Franco and Trapp (2008) model estimated low sorption would occur for nevirapine at both soil pH (Table 4.7). It was more successful at predicting the Log K_{oc} for the loam soil but the sandy loam was slightly over-predicted (by 0.1 mL g OC^{-1}). This success at predicting the sorption fate of nevirapine is probably due to the fact that it will be neutral due to the low pK_a value resulting in less complex

interactions with the soil making the simple semi-empirical model more reliable (Schaffer et al. 2012).

As with ofloxacin, changing the soil: solution ratio would allow for a more robust estimate of the sorption for naproxen and nevirapine. A greater amount of soil used would allow for more potential sorption sites for these APIs to interact with. The OECD 106 guideline recommends a soil: solution ratio of 1:1 can be used for chemicals that are not likely to sorb to soils (OECD 2000). A recommended minimum sorption of 20 % is recommended in this guideline.

The lack of sorption of nevirapine makes it more mobile in soils that ofloxacin or propranolol and results in the ability of the API to move into water bodies either from wastewater irrigation of fields or the discharge of effluent to streams. Nevirapine has been measured at high concentrations (0.06 – 5.6 μg L^{-1}) in groundwater and rivers in Kenya, where use of this antiretroviral in high (K'Oreje et al. 2016). It is persistent in these environments, with poor removal from wastewater treatment plants due to its low sorption potential, photostability and poor biodegradability (Prasse et al. 2010; K'Oreje et al. 2016; Aminot et al. 2018).

4.6.6 Changes to soil suspension properties over the sorption/desorption experiments

As has been discussed previously, the pH of soil suspensions influences the net charge on ionisable APIs and they will be fully ionised (> 99 %) when the pH is at ± 2 pH units from their pK_a (Lees et al. 2016). Variations in soil suspension pH over the length of the sorption experiment could therefore have an impact on the ionisation state of the APIs in question, which has been demonstrated for ofloxacin (other APIs are not affected at the experimental pH range) (Figure 4.1). Due to the rapid initial sorption of ofloxacin in this experiment, any influence pH change had over the ionisation state was

not observed in the results. Variation in pH could cause more significant changes to sorption behaviour in other compounds with a pK_a around 6 under these conditions. Soil pH will also influence the pH-dependent charge on the organic matter, clay minerals and metal sesquioxide components of the soil, which may influence API sorption (Hyun et al. 2004).

The DOC concentration increased over the 120 hour shaking period for both soils. Dissolved organic matter (DOM) has the potential to associate with APIs and therefore increase their mobility compared with those sorbed to soil particles (Tolls 2001). Organic matter is often determined by measurement of organic carbon; typically natural organic matter comprises 50 % carbon (Schwarzenbach et al. 1993). Soil suspensions contain large concentrations of water extractable DOC (3.6 – 44 mg L^{-1} for this study) (Figure 4.9) (12.1 – 27.2 mg L^{-1}, literature values) and varies significantly between soils (Maxin et al. 1995). Variation in DOC concentration may have affected the concentration of API assumed to be sorbed to the soil if it was not freely available for the analytical method in the suspension (Maxin et al. 1995).

The ability of DOM to bind organic contaminants has been reported since the 1980s but very few studies involving APIs have been reported for soil or wastewater DOM (Chiou et al. 1986; Chiou et al. 1987; Lees et al. 2016). It has been studied for the antibiotic ciprofloxacin, where DOC from humic material was compared with that from wastewater sources. Ciprofloxacin (pK_a 5.90, 8.89) was reported to partition into the DOC from humic sources more readily than from the wastewater source; this mechanism was pH dependant cation exchange (Carmosini et al. 2009).

Changes to soil suspension properties during the desorption experiment after replacement of 10 mM $CaCl_2$ solutions with fresh showed that the pH of the different soils were very similar (loam had decreased by 0.6 pH units) but the loam was more stable

over the 120 hour shaking period. As mentioned previously the scale of pH change in the loam soil could influence the fraction of API ionised at the experimental conditions affecting the desorption compared to sorption calculations.

4.6.7 Degradation pathways

Some of the loss of APIs from solution could have been the result of biotic or abiotic degradation (Hurtado et al. 2017). Figure 4.3 shows the percentage change of the APIs over time in just the 10 mM $CaCl_2$ solution. This indicates that all four APIs degraded or sorbed to the vessel walls to some extent during 120 hours shaking in the dark at room temperature. This decrease of API over time was not large enough to affect the amount of sorption observed when the soils were added to the experiment. Whilst this experiment was performed cleanly, it was not sterile and microorganisms may have been present in the RO water or the $CaCl_2$ powder initially.

Biotic degradation is driven by the activities of microorganisms in soils. These activities are dependent on many factors such as; O_2, pH, temperature, moisture levels, the population diversity of microorganisms, nutrients availability, chemical structure of the compound and cellular transport properties (Pan et al. 2017). Sterilising the soils in the current study was unsuccessful (Chapter 3). As a result it is not possible to identify how much of the loss from solution was due to biotic degradation (Lees et al. 2018). To identify possible biodegradation in soils the same experiment discussed in Section 4.4.2 can be repeated but with sterile soils and the loss of API from the liquid phase can be compared (Al-Rajab et al. 2010).

Abiotic degradation processes, i.e. those that do not involve microorganisms, which can occur in soils include hydrolysis and photolysis (Hurtado et al. 2017). In other environmental compartments, oxidation and reduction may be important but there is a lack of data on this as a possible degradation process occurring in soils (Jiménez et al.

2016). Hydrolysis is the process of degrading an API by breaking a bond in a molecule using water. It can be measured using the OECD 111 guideline but this does not include soil in the method (OECD 2004). This reaction is controlled by environmental parameters with pH and temperature being the most important (ECB 2002; Mitchell et al. 2014). Photolysis is the process of degrading an API by UV-light. This can be measured by exposing a solution of API to UV light for a period of time with a control sample handled identically that had been stored in amber colours vials (OECD 2008; Vulava et al. 2016). The risk of photolysis was removed from the present study by wrapping all test vessels in aluminium foil during the shaking process and storing all samples and standards in the dark. It appears that much more work has been done in environmental waters rather than soils for all kinds of abiotic degradation.

Degradation and sorption of APIs in soils can be described using half-lives, which is the time it takes for half of the initial soil concentration to decrease by 50 %. Literature data for the APIs in this study vary significantly. Ofloxacin has reported half-lives of <110-1500 days, propranolol >40 days, and naproxen 5.9-69.3 days (Tables 2.2, 2.4 and 2.7). No data was available for nevirapine in literature. Differences between API half-lives are probably the result of differences in soil properties such as moisture content, OC, pH, microbial activity, temperature as well as physico-chemical properties of the API such as proportion of ionisation and lipophilicity (Monteiro et al. 2009). Half-lives give an indication of how long an API is considered to be present in the soil for. This is useful to know as it can help develop risk assessments for soil dwelling organisms by predicting more accurate PEC_{soil} values (Walters et al. 2010; Bourdat-Deschamps et al. 2017).

4.7 Conclusions

The aim of this chapter was to use the OECD 106 method to assess the loss of APIs from soil suspensions and suggest the possible physico-chemical mechanisms controlling the partitioning processes. The four APIs studied behaved differently in the soils.

The overall driver of the differences between the soils was the ionisation state of the APIs. Propranolol, which was positively charged at the experimental pH, showed strong sorption capacity for the loam soil, which had the greatest organic carbon content and clay content (negatively charged sites). Ofloxacin, a zwitterion in these soil samples, had the potential for many different sorption mechanisms and sorption sites, which was identified by its strong and mostly irreversible sorption to both soils. Naproxen had a negative charge promoting charge repulsion to most soil surfaces so less sorption occurred but negatively-charged compounds can interact with organic matter, evidenced by a greater K_d in the loam soil. The neutral API nevirapine showed very little sorption in both soils but what occurred was due to hydrophobic interactions to the organic matter in the soils, again shown by greater sorption in the loam soil. Organic matter in soils is one of the larger drivers controlling the fate of APIs in soils, but due to a lack of consistency in the Log K_{oc} data between the two soils, there must be another important sorption site that should be included as well. Clay generally has a high CEC allowing for sorption of positively-charged APIs, there is also the potential for some clays to have an anion exchange capacity due to protonation of the surface hydroxyl groups (Lees et al. 2016). The importance of clay sorption depends on the environmental pH, API pK_a and anion : cation exchange capacity ratio. Whilst this study is limited to two soils and four APIs it showed the large differences in sorption that can occur as a results of differences in APIs and soil characteristics.

The use of the Franco and Trapp (2008) model to predict the Log K_{oc} of these compounds was successful for naproxen and nevirapine. For the APIs that showed high sorption (propranolol and ofloxacin) this model was not accurate enough. This suggests that better models which are mechanistic and process-based that consider relevent boundary conditions are required (Schaffer et al. 2012). The more complex artificial neural networks developed by Barron et al (2009) may be able to successfully predict the sorption fate of these APIs but more computing power would be required for these to be successfully run along with a large training data set.

The diversity of chemical characteristics of APIs and soil properties across LLMICs makes the challenge of producing robust environmental risk assessments globally difficult. More studies with a larger amount of APIs and soils that have been sourced from LLMICs are needed that are performed in a comparable manner to add to a global dataset that will solve these challenges.

5 API loss from synthetic wastewater to soils

5.1 Overview

This chapter investigates the fate of four APIs in two soil suspensions that had synthetic wastewater (SWW) added instead of 10 mM $CaCl_2$. The aim of this study was to understand how adding SWW to soils effects the soil suspension properties and how this impacts the fate of the APIs in soils.

The main findings of this chapter was that SWW alters soil properties and that this, in turn, can affect the extent of API sorption to soils. Irrigation with wastewater changes some of the soil properties important for chemical fate (pH, organic matter content and addition of microorganisms). DOC from SWW was measured and a loss from the suspensions was identified. This loss of SWW DOC over the length of the loss from suspension experiment possibly can increase the partitioning of positively charged APIs, this was apparent for propranolol in sandy loam.

The ionisation state of the API at the altered pH after irrigation was more important for the positively charged propranolol than it was for the negatively charged naproxen and neutral nevirapine. In some cases the addition of SWW increased the loss from solution and therefore increased K_d and Log K_{oc} during the 120 hour shaking experiment. This has implications on the current terrestrial risk assessment where the trigger value for a more detailed soil risk assessment is Log $K_{oc} > 4$. If the experiment is only performed in 10 mM $CaCl_2$, as is currently required, it could lead to unknown risks of APIs in wastewater irrigated soils not being taken into account.

5.2 Introduction

The use of wastewater for irrigation of agricultural soils happens globally (Christou et al. 2017). The input of wastewater to soils has numerous benefits for farmers and communities in LLMICs experiencing water shortages and it is sometimes used as a tertiary treatment process (Durán-Alvarez et al. 2009; Lees et al. 2016). Benefits include freeing up freshwater sources for drinking, preventing untreated wastewater from entering rivers and an additional nutrient input to soils (e.g. carbon, phosphorus and nitrogen) (Scott et al. 2004; Gibson et al. 2010). Problems associated with this practice include the introduction of pathogens, bacteria or viruses, trace organics, heavy metals and other pollutants, as well as potentially increasing the salinity of soils (Toze 2006). Concentrations of APIs that have been found in treated and untreated wastewater vary globally within country and within treatment plant (Table 5.1). High concentrations of certain APIs are not confined to LLMICs after treatment, as shown by naproxen in UK effluents where up to 1.11 $\mu g\ L^{-1}$ have been measured. Table 5.1 suggests that even where wastewater treatment has been used there may be levels of APIs entering either surface water environments or soils via irrigation that may pose hazards to flora and fauna within those compartments.

Table 5.1 – Examples of concentrations of APIs in wastewater influents and effluents globally

API	Country	Country dev. level*	Conc. pre-treatment (μg L^{-1})	Conc. post-treatment (μg L^{-1})	Reference
Atenolol	India	LMI	5.2-41.4		(Mohapatra et al. 2016)
Atenolol	India	LMI	1.4-2.9	0.6-1.5	(Subedi et al. 2017)
Atenolol	UK	HI	1.11-2.22	0.24-0.41	(Nakada et al. 2017)
Carbamazepine	India	LMI	1.5-18.5		(Mohapatra et al. 2016)
Carbamazepine	India	LMI	0.45-0.55	0.48-0.58	(Subedi et al. 2017)
Carbamazepine	Kenya	LMI	ND-0.35	ND-0.32	(K'Oreje et al. 2016)
Carbamazepine	UK	HI	0.28-0.79	0.27-0.88	(Nakada et al. 2017)
Ciprofloxacin	India	LMI	69.8-246.1		(Mohapatra et al. 2016)
Diclofenac	Kenya	LMI	0.93-1.51	0.03-0.06	(K'Oreje et al. 2016)
Ibuprofen	India	LMI	1.2-1.4	0.63-0.98	(Subedi et al. 2017)
Ibuprofen	Japan	HI	0.69-1.05	0.01-0.06	(Nakada et al. 2006)
Ibuprofen	Kenya	LMI	6.46-10.55	ND-2.07	(K'Oreje et al. 2016)
Naproxen	Japan	HI	0.08-0.23	0.01-0.06	(Nakada et al. 2006)
Naproxen	UK	HI	3.8-8.92	0.26-1.11	(Nakada et al. 2017)
Nevirapine	Kenya	LMI	0.85-3.30	1.03-2.11	(K'Oreje et al. 2016)
Norfloxacin	India	LMI	0-25.3		(Mohapatra et al. 2016)
Propranolol	India	LMI	0.04-0.05	0.03-0.04	(Subedi et al. 2017)
Triclosan	Japan	HI	0.30-0.62	0.08-0.26	Nakada et al 2006

Dev = development, ND = no detect (<LOD), LMI = lower middle income country, HI = high income country

*Classification based on World Bank data (The World Bank 2018)

Wastewater carries many different contaminants, including APIs, which at elevated concentrations can have negative impacts on soil ecosystems or other linked environmental compartments (such as groundwater). Understanding the changes to soil properties that wastewater irrigation causes, and how this impacts chemical fate, will aid the development of terrestrial risk assessments as it is currently not considered (Lees et al. 2016). DOC in wastewater at mg L^{-1} levels could influence the partitioning characteristics of APIs. Either by stabilising APIs in the liquid phase and increasing

mobility or complexing and partitioning to soil via carbon-carbon interactions (Müller et al. 2007).

Wastewater characteristics vary globally and depend heavily on the inputs into the sewage network Table 5.2 (Metcalf et al. 2002). It is very difficult to replicate this complex matrix within the laboratory setting due to this inherent variability (Bagnis et al. 2018). SWW was chosen for the following experiment rather than raw wastewater to ensure that results were repeatable and conditions could be controlled, as the composition of wastewater varies in the same WWTP from day to day and also spatially (Metcalf et al. 2014). SWW is commonly used for experiments to identify the fate of chemicals in environmental matrices, ecotoxicology studies, in the evaluation of new treatment processes and in many other areas of research (Jiang 2007; Paul et al. 2013; Ma et al. 2017). The use of SWW reduces the potential of APIs being present in the matrix as only a small proportion of the matrix is primary sludge and this had been washed prior to use. pH of untreated wastewater tends to be in the range of 6.8-7.4 and the DOC in high income countries has been shown to be around 49-72 mg L^{-1} (Table 5.2) so these characteristics were replicated in the SWW used for the following experiments.

Table 5.2 – Examples of wastewater influent (untreated) characteristics

Country	Country dev. level	pH	DO	TOC	DOC	BOD	COD	TSS	Ref.
Australia	HI				49				(Neale et al. 2011)
Greece	HI				72				(Katsoyiannis et al. 2007)
Bolivia	LMI	7.4				395	786		(Zabalaga et al. 2007)
India	LMI	7.4	0.7	9.6		107		175.7	(Mohapatra et al. 2016)
India	LMI	6.8	2.6	17.7		79		168.0	(Mohapatra et al. 2016)
India	LMI	8.4	2.4			620	1421	1824.0	(Kumar et al. 2012)
Kenya	LMI	7.2				615	1128	693.0	(K'Oreje et al. 2016)
Kenya	LMI	7.1				1749	3200	260.0	(K'Oreje et al. 2016)
Kenya	LMI	7.4				213	390	390.0	(K'Oreje et al. 2016)
Pakistan	LMI	6.9	2.7						(Ensink et al. 2002)

DO = dissolved oxygen (mg L^{-1}), TOC = total organic carbon (mg L^{-1}), DOC = dissolved organic carbon (mg L^{-1}), BOD = biological oxygen demand (mg L^{-1}), COD = chemical oxygen demand (mg L^{-1}), TSS = total suspended solids (mg L^{-1}). Country development level classed the same as Table 5.1.

The aim of this chapter was to use SWW instead of 10 mM $CaCl_2$ in the OECD 106 experiment and compare the results with those gained in Chapter 4. This data provided information on the impacts of irrigating with wastewater has on the sorption fate of APIs in soils. Data from this chapter was used to understand the need of improved risk assessments that include wastewater irrigation as a source of APIs to soil.

5.3 Methods

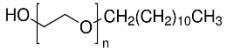
5.3.1 Synthetic wastewater matrix

A formulation of SWW was chosen that closely resembled real wastewater, included primary sludge (Central Wastewater Treatment Works, Plymouth, UK) and had been used in other studies (Boeije et al. 1999; O'Flaherty et al. 2013; Bagnis et al. 2018).

The primary sludge was washed, freeze dried and deactivated at 103 °C before being added to the SWW mixture following a standard method (US EPA 1996). The washing removed any colour, matrix materials and water soluble compounds before use. This was done by shaking approximately 10 g of primary sludge with 30 mL HPW in 50 mL polypropylene centrifuge tubes before centrifuging (5 minutes, 4000 RPM) and removing supernatant. This washing step was performed three times before freeze drying. Freeze drying of the washed sludge was performed to destroy microorganisms present in the sludge. The washed sludge was frozen at -20 °C overnight and put in the freeze dryer, with the lids of the centrifuge tubes loosely fastened, for 30.5 hours. The sludge was then deactivated to ensure that microbes surviving freeze drying were killed. This was achieved by placing the freeze dried sludge in glass beakers and transferring to a furnace at 103 °C for 15 hours. The deactivated sludge was stored at 4 °C in sterile polypropylene centrifuge tubes until use (one month).

The SWW was made in a 1 L beaker and stirred in the dark on a magnetic stirrer for 24 hours, it was made at 25 times the concentration required for the experiments. To remove particulates in the SWW that may compete with soil particles for APIs, the SWW was filtered using 0.7 μm glass fibre filters (ashed). Whilst in a real world irrigation setting there will be particulates in the wastewater (Table 5.2) filtering the SWW removed an element of uncertainty in discussing the results from this experiment. It was then stored at -20 °C until needed and then diluted to the required strength before use (Boeije et al. 1999). The experimental concentrations and details of components used are described in Table 5.3.

Table 5.3 - Synthetic wastewater components (Boeije et al. 1999; Bagnis et al. 2018)

Source of*	Compound	Chemical formula	Supplier	CAS	Concentration (mg L^{-1})
C	Sodium acetate	$C_2H_3NaO_2$	Fisher Scientific	127-09-3	33.33
C	Meat extract		Fluka Analytical		15.00
C	Lactose monohydrate	$C_{12}H_{22}O_{11}\cdot H_2O$	Fisher Scientific	63-42-3	33.33
C	Potato starch	$(C_6H_{10}O_5)n$	Acros Organics	9005-25-8	33.33
C	Glycerol	$C_3H_8O_3$	Acros Organics	56-81-5	20.00
C	Peptone		Fluka Analytical	91079-38-8	28.33
N	Ammonium chloride	NH_4Cl	Fisher Scientific	12125-02-9	11.00
N	Urea	CH_4N_2O	Fisher Scientific	57-13-6	75.00
N	Uric acid	$C_5H_4N_4O_3$	Acros Organics	69-93-2	9.00
P	Potassium phosphate monobasic	KH_2PO_4	Fisher Scientific	7778-77-0	20.00
	Magnesium sulfate heptahydrate	$MgSO_4\cdot 7H_2O$	Fisher Scientific	10034-99-8	25.00
Sewage simulation	Genapol® C-100		Sigma Life Science	61791-13-7	3.33
Sewage simulation	Kieselguhr, pure white	O_2Si	Fisher Scientific	61790-53-2	10.00
Sewage simulation	Dextrin	$(C_6H_{10}O_5)x$	Acros Organics	9004-53-9	33.33
Sewage simulation	Genapol® X-080	$HO(CH_2CH_2O)_n(CH_2)_mH$	Sigma Life Science	9043-30-5	3.33
Sewage simulation	Lyophilized activated sludge				200.00

Minerals and trace metals	Calcium chloride dihydrate	$CaCl_2 \cdot 2H_2O$	Fisher Scientific	10035-04-8	5.00
Minerals and trace metals	Sodium bicarbonate	$CHNaO_3$	Acros Organics	144-55-8	25.00
Minerals and trace metals	Iron(III) sulphate hydrate	$Fe_2O_{12}S_3 \cdot 5H_2O$	Fisher Scientific	15244-10-7	10.00
Minerals and trace metals	Cobalt(II) chloride hexahydrate	$CoCl_2 \cdot 6H_2O$	Acros Organics	7791-13-1	0.05
Minerals and trace metals	Chromium(III) nitrate nonahydrate	$Cr(NO_3) \cdot 9H_2O$	Acros Organics	7789-02-8	0.68
Minerals and trace metals	Copper(II) chloride dihydrate	$CuCl_2 \cdot 2H_2O$	Fisher Scientific	10125-13-0	0.48
Minerals and trace metals	Ethylenediaminetetraacetic acid (EDTA)	$C_{10}H_{16}N_2O_8$	Fisher Scientific	60-00-4	0.22
Minerals and trace metals	Potassium molybdate	K_2MoO_4	Aldrich chemistry	13446-49-6	0.02
Minerals and trace metals	Manganese(II) sulphate monohydrate	$MnSO_4 \cdot H_2O$	Acros Organics	10034-96-5	0.10
Minerals and trace metals	Nickel(II) sulphate hexahydrate	$NiSO_4 \cdot 6H_2O$	Acros Organics	10101-97-0	0.30
Minerals and trace metals	Zinc chloride anhydrous	$ZnCl_2$	Acros Organics	7646-85-7	0.18
Buffer	Sodium dihydrogen orthophosphate dihydrate	$H_2NaO_4P \cdot 2H_2O$	Fisher Scientific	13472-35-0	259.60
Buffer	Disodium hydrogen orthophosphate anhydrous	HNa_2O_4P	BDH Chemicals	7558-79-4	2176.40

*C – carbon, N – nitrogen, P – phosphorus

5.3.2 Preliminary experiments

5.3.2.1 Matrix effects

To ensure that the addition of SWW to the soil suspensions did not affect the analytical method the matrix was spiked with a range of concentrations of API and the recovery determined. This was done according to the method discussed in Chapter 2. Blank matrix samples were also included to ensure that the SWW did not include any quantifiable residues of APIs.

5.3.2.2 Characterisation of humic and fulvic components of SWW and soils

This was undertaken following the method described in Chapter 2. 6 g of soil with 30 mL 10 mM $CaCl_2$ or SWW were shaken overnight before centrifuging (4000 RPM, 15 minutes) and filtering (0.7 µm GFF). Samples (2 mL) were added to a quartz glass cuvette and analysed using 3-D fluorescence spectrophotometry (excitation 200-400 nm, emission 280-540 nm). The results were compared with reported values to identify humic and fulvic components in soils with 10 mM $CaCl_2$ and with SWW (Chen et al. 2003). The ratio of soil : solution was consistent with all other experiments (1:5).

5.3.2.3 API stability in SWW

To ensure that the APIs were stable in SWW soil suspensions during the entire experiment, APIs (Table 5.4) were added to 30 mL SWW in 50 mL polypropylene centrifuge tubes and shaken for 120 hours at room temperature (15 – 20 °C). Sample tubes were sacrificed at the same time points as the 120 hour sorption experiment before analysis using HPLC-HRAM-MS method developed previously.

Table 5.4 - Spike concentrations of APIs

API	Spike concentration (µg L^{-1})
Ofloxacin	500
Propranolol	200
Naproxen	30
Nevirapine	50

5.3.3 Sorption in soils treated with SWW

The 120 hours sorption experiment was carried out in the same way as that without SWW. Briefly, a 6 g aliquot of soil was added to 50 mL polypropylene centrifuge tubes along with 30 mL of SWW in triplicate for each time point. These were put onto a shaker laid horizontally to allow the system to equilibrate overnight. The tubes were then spiked and returned to the shaker (Table 5.4). Tubes were sacrificed at pre-selected time periods after spiking (0, 3, 6, 24, 48, 72, 96, 120 hours), centrifuged (4000 RPM, 15 minutes) then filtered using 0.7 µm glass fibre filters. Samples were then stored at -20 °C until analysis, along with calibration solutions and matrix blanks prepared in parallel. Analysis was performed by HPLC-HRAM-MS as described in Chapter 2. This experiment was carried out at room temperature (15 - 20 °C) and in the dark (tubes wrapped in aluminium foil) to ensure that photodegradation of the APIs did not occur.

The pH and DOC concentration was measured at all the time points in centrifuge tubes without APIs spiked in them. The methods followed are described in Chapter 2. From the concentrations measured in the solution, assuming that the rest of the added API had sorbed to the soil after being corrected for loss to filter papers, the K_d, Log K_{oc} and percentage loss could be calculated for each API as described in Chapter 4.

5.4 Results

5.4.1 Preliminary experiments

5.4.1.1 Matrix effects

The results from this preliminary experiment indicated that quenching was observed but the corresponding calibration was linear, matrix-matched calibrations were used to quantify the APIs. More detail can be found in Chapter 2. Blank SWW did not contain any quantifiable residues of the APIs used in this experiment.

5.4.1.2 Stability of APIs in SWW

Shaking the API-containing SWW for 120 hours did not lead to degradation of propranolol, naproxen and nevirapine (Figure 5.1). There was interference of the signal for ofloxacin across all data points so the results are not shown. Another compound was co-eluting from the HPLC column at the same time making peaks unquantifiable. Limited availability of the HPLC-HRAM-MS meant that this issue could not be resolved. As a result there was no further testing of ofloxacin under these conditions.

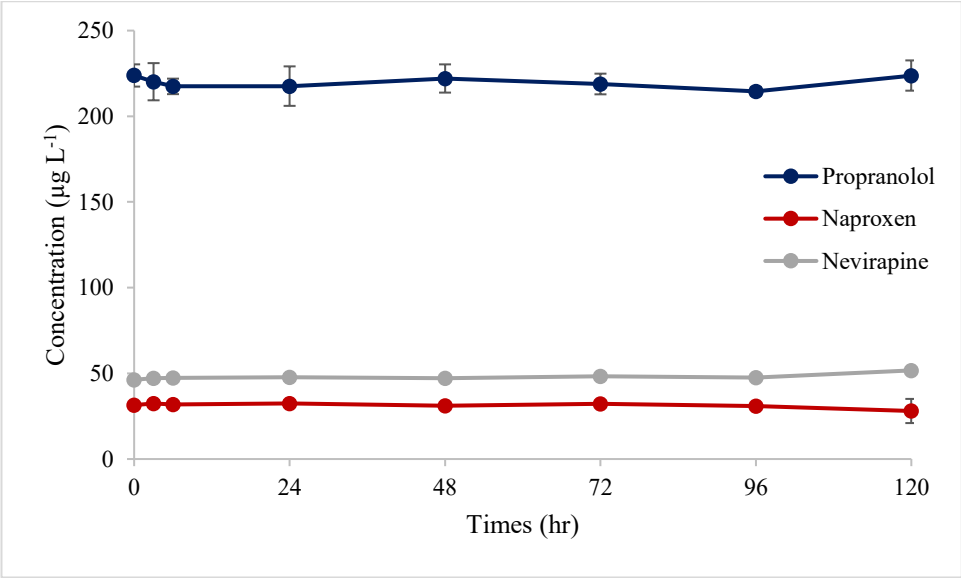


Figure 5.1 - Stability of APIs in wastewater containing APIs over 120 hours shaking ($\bar{x}$ ± S.D. n = 3). Error bars are present for all data points but not visible in some cases.

5.4.1.3 Humic and fulvic components

The SWW fluorescence spectrum contained two peaks, characteristic of aromatic proteins and tryptophan-soluble microbial by-products, proving confidence that SWW can be used as a good replacement of wastewater (Chen et al. 2003; Hernandez-Ruiz et al. 2012). The analysis of the humic and fulvic components of soil filtrates using 3-D fluorescence with and without SWW showed that the addition of SWW resulted in suppression (quenching) of the overall fluorescence of the organic matter matrix (typically loss of the peaks for aromatic proteins and tryptophan-soluble microbial by-products) (Figure 5.2). The loam soil showed little difference following addition of SWW (Table 5.5 and Figure 5.2) and the peaks were characteristic of humic and fulvic acids, based on library spectral comparisons (Figure 5.3). The sandy loam sample spectrum also contained peaks that were humic and fulvic acid like. None of the biological or protein-like peaks were identified in either soils after addition of SWW. The peak intensity varied considerably between matrix types. The loam soil with 10 mM $CaCl_2$ had the strongest intensity and the sandy loam with the SWW the lowest (Table 5.5).

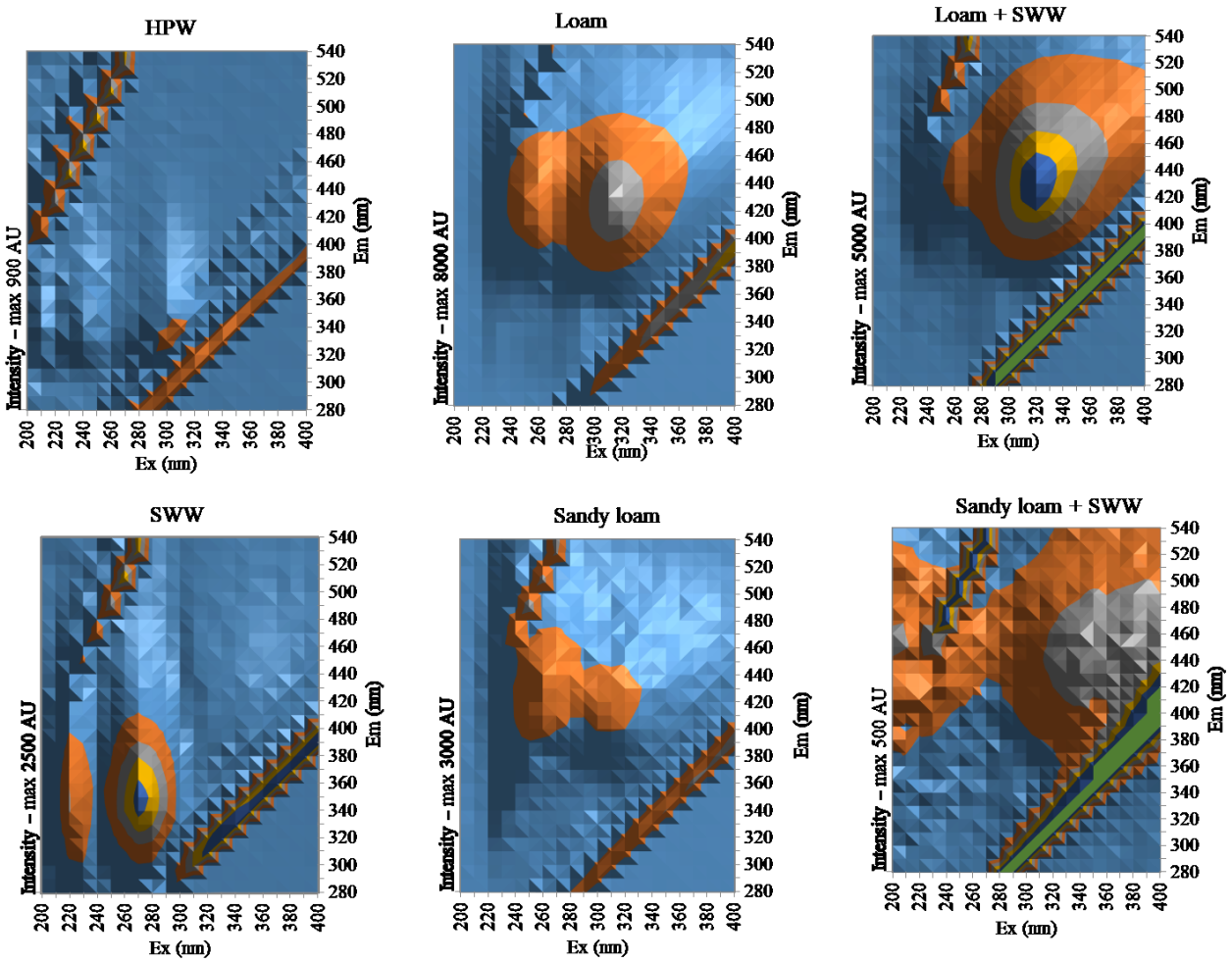


Figure 5.2- 3D Fluorescence results of different soil and SWW wastewater matrices (Ex – excitation, Em – emission)

Table 5.5 - Peak intensities (AU) for matrices using 3D fluorescence spectrophotometry

Peak	Figure 5.3 letter	SWW	Loam	Loam + SWW	Sandy loam	Sandy loam + SWW
EX270 EM350	A	2245				
EX220 EM340	B	1022				
EX320 EM430	C		5440	4873		
EX250 EM440	D		3090	1193		
EX260 EM440	E				1517	
EX300 EM420	F				1116	
EX370 EM450	G					269

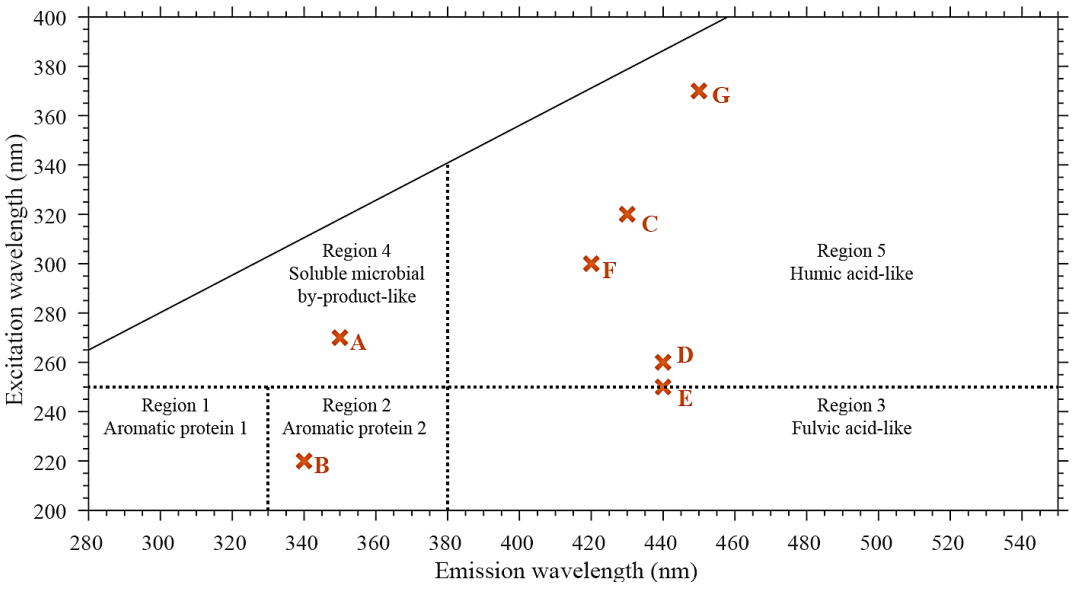


Figure 5.3 - Location of excitation and emission peaks of components identified in matrices (orange crosses). Figure modified from Chen (2003)

5.4.2 Matrix changes in soil suspensions with SWW

The pH of the suspensions varied with and without SWW when shaken over time (Figure 5.4). The SWW pH mainly stayed around pH 7.0. The dip in pH at 96 hours is an experimental error probably caused by an issue with the pH probe. In loam with SWW, the pH significantly increased from 6.9 to 7.2 over 48 hours (*t*-test, unequal variances, two-tailed $p \leq 0.05$) but was stable after this. Whereas, as discussed in Chapter 4, the pH continuously decreased in 10 mM $CaCl_2$ solutions. Sandy loam soils had a similar pattern, excepting a slightly higher pH in SWW suspensions. The SWW pH was 6.6 and decreased slightly over 6 hours then significantly increased to 7.0 after 72 hours of shaking (*t*-test, unequal variances, two-tailed $p \leq 0.05$).

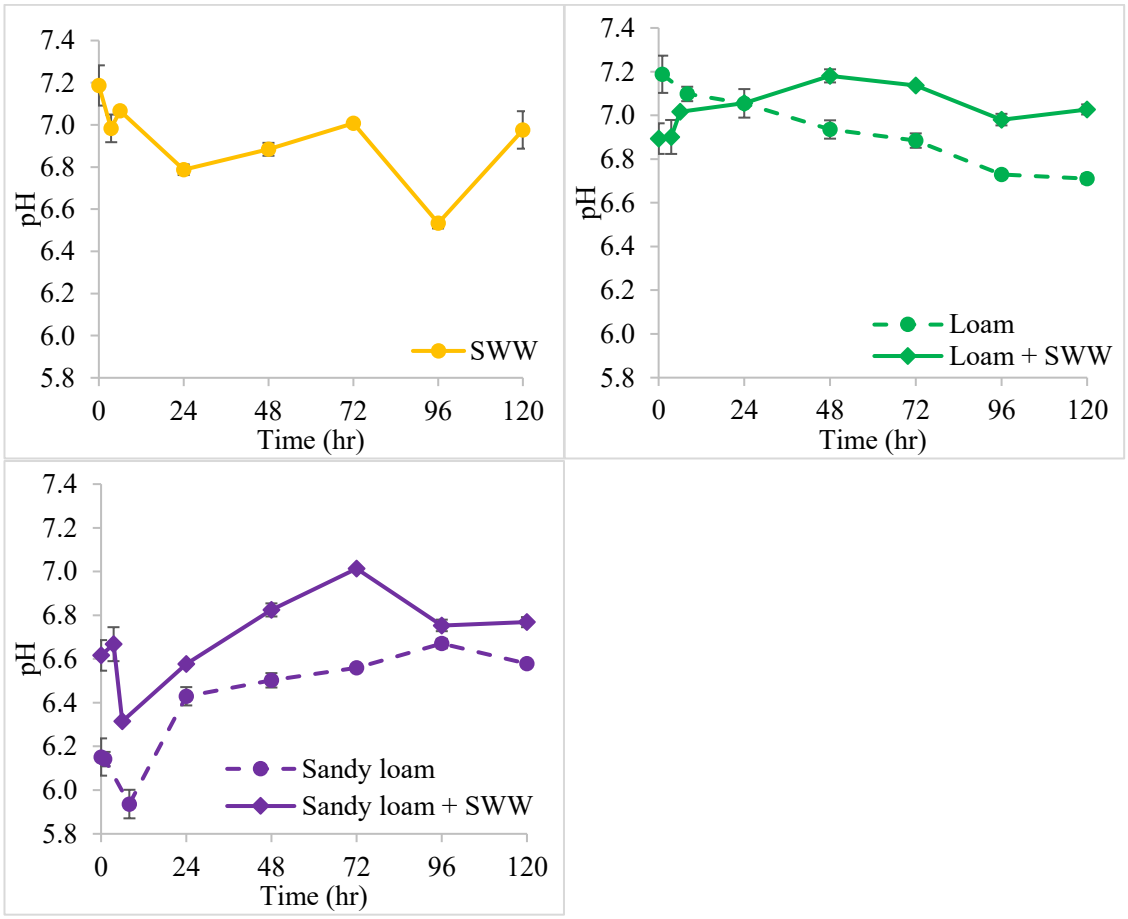


Figure 5.4 - pH in soil suspensions in SWW and 10 mM $CaCl_2$ ($\bar{x}$ ± S.D. n = 3). 0 hr is after system equilibrated overnight. Error bars are present for all data points but not visible in some cases.

All DOC concentrations in SWW matrices showed a general trend of decreasing DOC concentration throughout the shaking time and contrasted with the soil solution in 10 mM $CaCl_2$, where a general increase over time was measured (Figure 5.5). DOC concentrations in SWW significantly decreased from 73 to 36 mg L^{-1} over 72 hours (*t*-test, unequal variances, two-tailed $p \leq 0.05$) before increasing to 36 mg L^{-1} at the end of the experiment. This decrease was reflected in the loam soil with SWW which started at 93 mg L^{-1} before decreasing to 60 mg L^{-1} at 72 hours (*t*-test, unequal variances, two-tailed $p \leq 0.05$). The addition of SWW increased the DOC concentration in loam filtrate by 62 mg L^{-1} initially, almost accounting for the SWW DOC concentration. This contrasts with the sandy loam solution where the addition of SWW only caused an increase of 43 mg L^{-1}.

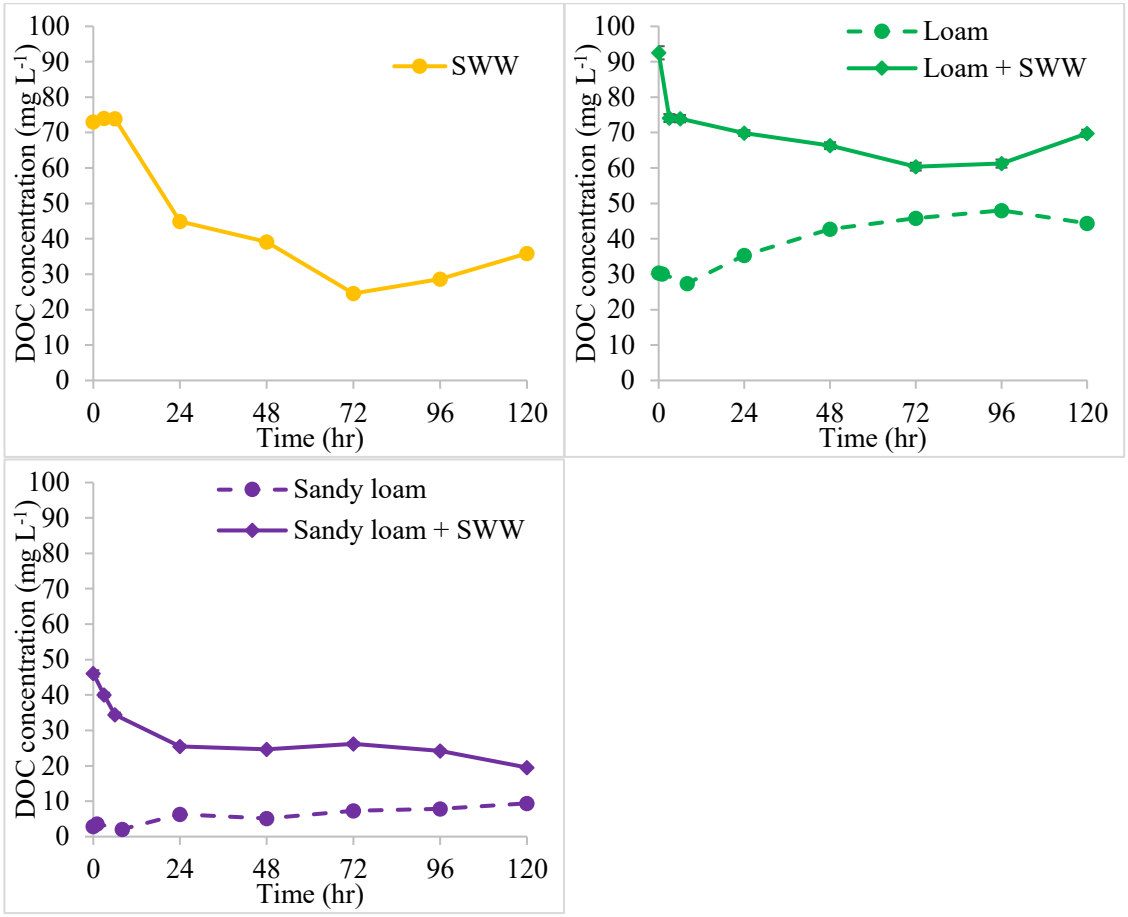


Figure 5.5 - DOC of soil filtrates in SWW and 10 mM $CaCl_2$ ($\bar{x}$ ± S.D. n = 3). 0 hr is after system equilibrated overnight. Error bars are present for all data points but not visible in some cases.

5.4.3 Sorption experiment in SWW

More API was lost from suspension in soils with SWW added compared with soils suspensions containing 10 mM $CaCl_2$ (Figure 5.6). Percentage loss from suspension was used to compare differences in sorption behaviour as direct comparison of APIs in soils containing SWW could not be compared with in 10 mM $CaCl_2$ soil solutions due to variations in spike concentration and the fact that the experiments were performed six months apart.

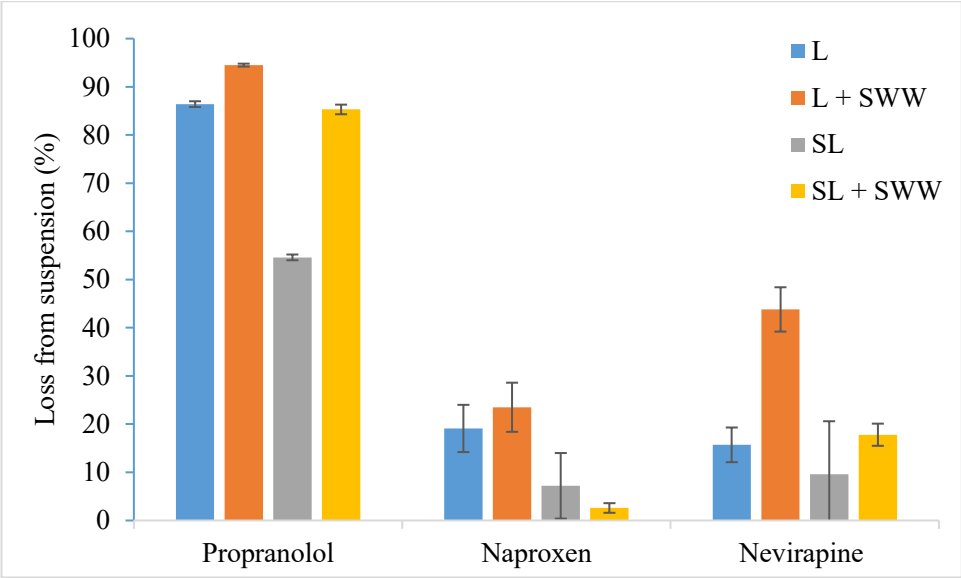


Figure 5.6 - Loss from soil suspension at equilibrium with SWW compared to 10 mM $CaCl_2$ ($\bar{x}$ ± S.D. n = 4 - 6).

Propranolol in the sandy loam showed the greatest difference in loss from suspension at equilibrium between the two solution matrices (Figure 5.6). There was a significant difference between the loss from suspension in the two matrices for both soils at equilibrium (*t*-test two sample assuming equal variance, $p \leq 0.05$). The changes in loss from suspension over time between the two matrices were similar where there was an initial rapid increase in loss up to 6 hours then it levelled out before reaching equilibrium (Figure **5.7**). The changes in loss from suspension was reflected by the differences between K_d and Log K_{oc} between the two solution types in the same soil; these were increased for suspensions containing SWW (Table 5.6). The time to reach equilibrium was impacted by the different matrices but it was not consistent across soils. Loam with SWW reached equilibrium at 48 hours instead of 72 hours but in sandy loam to equilibrium was delayed until 96 hours compared with 48 hours in $CaCl_2$.

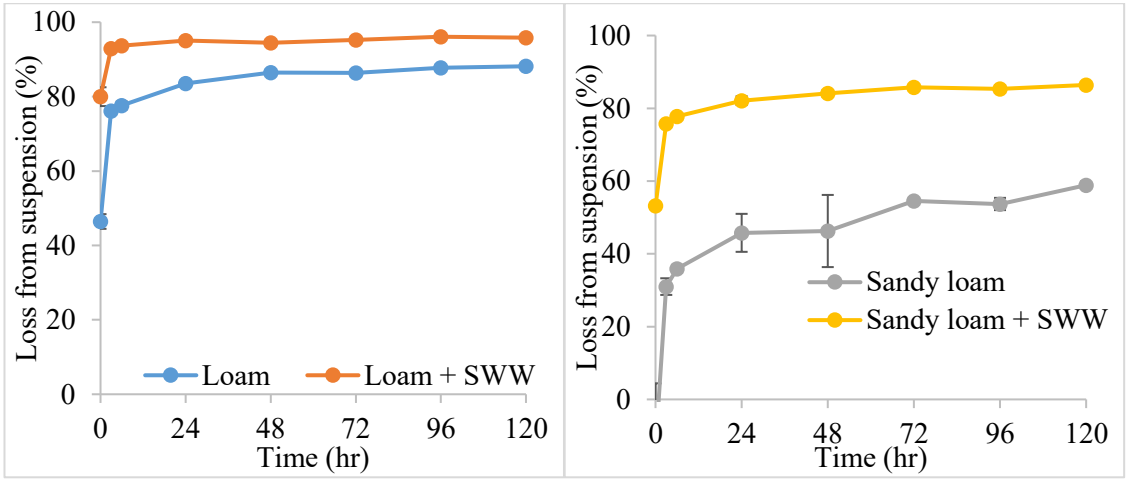


Figure 5.7 - Propranolol loss from suspension in soils with SWW and 10 mM $CaCl_2$ ($\bar{x}$ ± S.D. n = 4 - 6). Error bars are present for all data points but not visible in some cases.

Table 5.6 - Sorption data at equilibrium comparing soils with 10 mM $CaCl_2$ with SWW ($\bar{x}$ ± S.D. n = 4 - 6)

	Soil	Time to equilibrium (hr)	Suspension concentration (µg L^{-1})	Loss from suspension (%)	K_d (mL g^{-1})	Log K_{oc} (mL g OC^{-1})
Propranolol	L	72	27.3 ± 1.2	86.4 ± 0.6	32.8 ± 1.6	3.2 ± 0.02
Naproxen	L	48	33.9 ± 2.0	19.1 ± 4.9	1.4 ± 0.2	1.8 ± 0.1
Nevirapine	L	72	44.4 ± 1.9	15.7 ± 3.6	1.4 ± 0.3	1.9 ± 0.1
Propranolol	L + SWW	48	11.0 ± 0.6	94.5 ± 0.3	86.1 ± 4.9	3.6 ± 0.03
Naproxen	L + SWW	72	26.0 ± 1.7	23.5 ± 5.1	1.6 ± 0.4	1.9 ± 0.01
Nevirapine	L + SWW	72	32.0 ± 2.6	43.8 ± 4.6	4.0 ± 0.7	2.3 ± 0.04
Propranolol	SL	48	107.4 ± 19.8	54.6 ± 0.6	6.0 ± 0.1	3.0 ± 0.03
Naproxen	SL	24	38.3 ± 2.8	7.2 ± 6.8	0.4 ± 0.4	1.5 ± 0.7
Nevirapine	SL	48	57.8 ± 7.0	9.6 ± 11.0	0.3 ± 0.1	1.6 ± 0.1
Propranolol	SL + SWW	96	29.4 ± 0.3	85.3 ± 1.0	30.1 ± 0.4	3.7 ± 0.03
Naproxen	SL + SWW	24	33.1 ± 0.3	2.6 ± 1.0	0.1 ± 0.1	1.3 ± 0.2
Nevirapine	SL + SWW	3	46.9 ± 1.3	17.8 ± 2.3	1.1 ± 0.2	2.2 ± 0.1

L – loam, SWW – synthetic wastewater, SL – sandy loam

Naproxen showed similar patterns of loss from suspension over time with and without the SWW (Figure 5.8). For both soils there was no significant difference between the loss from both solution matrices at equilibrium (*t*-test two sample assuming equal variance, $p \leq 0.05$). The equilibrium times did not change in the sandy loam soil (24 hours) but it took longer for equilibrium to be reached in loam soils with SWW (48 hours with and 72 hours with SWW) (Table 5.6).

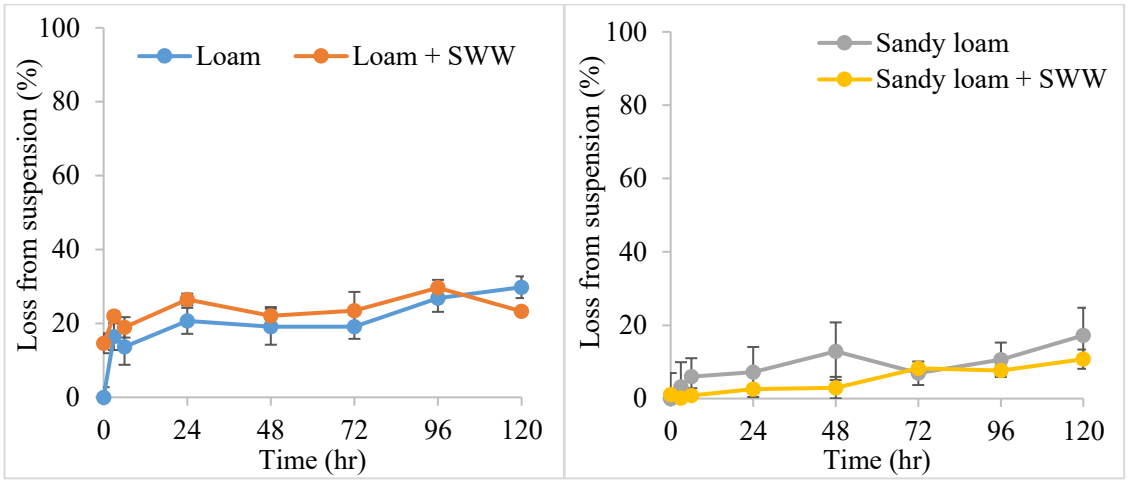


Figure 5.8 – Naproxen loss from suspension in soils with SWW and 10 mM $CaCl_2$ ($\bar{x}$ ± S.D. n = 4 - 6). Error bars are present for all data points but not visible in some cases.

Loss of nevirapine in loam soil with SWW suspension was significantly different at equilibrium to that in 10 mM $CaCl_2$ whereas there was no significant difference in the sandy loam (*t*-test two sample assuming equal variance, $p \leq 0.05$). Loam soils showed an initial rapid increase in loss before stabilising after 3 hours and sandy loam samples showed only very small losses from suspension, leading to a rapid equilibration time (3 hours) for this soil with SWW (Figure 5.9). There was no change in the time needed to reach equilibrium for the loam soils (72 hours).

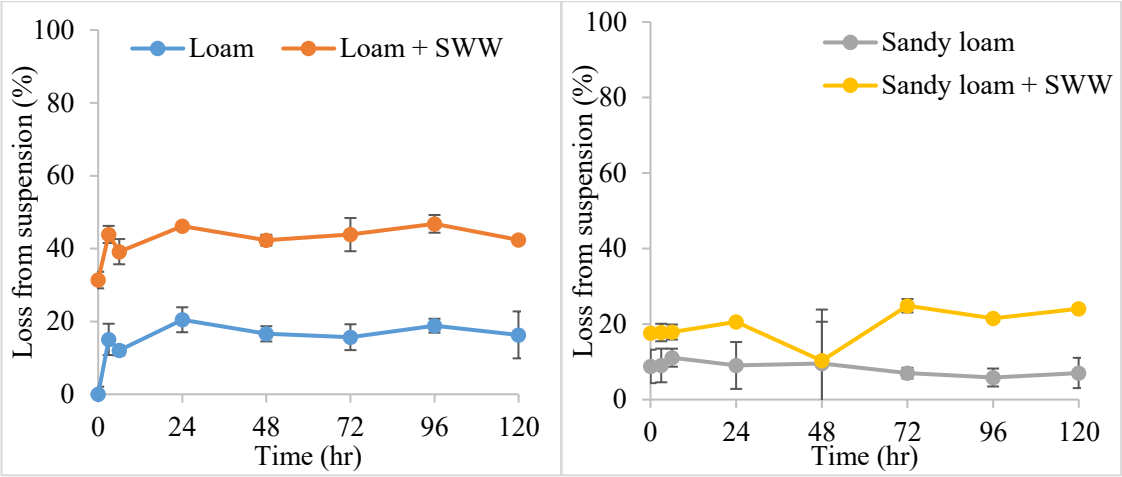


Figure 5.9 – Nevirapine loss from suspension in soils with SWW and 10 mM $CaCl_2$ ($\bar{x}$ ± S.D. n = 4 - 6). Error bars are present for all data points but not visible in some cases.

5.5 Discussion

5.5.1 Matrix changes

The addition of SWW to soils led to differences in the liquid phase during the 120 hour shaking experiment compared with the same experiments using 10 mM $CaCl_2$ solution. The SWW appeared to contained aromatic proteins and soluble microbial by-products (Figure 5.2) which have been identified in real wastewater showing that this SWW matrix can be used as a stable, reproducible surrogate for wastewater (Chen et al. 2003; Hernandez-Ruiz et al. 2012). These peaks in SWW were quenched in the soil filtrates and not visible in the results and also the loam and sandy loam soil peaks are quenched as well (Table 5.5). This quenching could be due to the SWW fractions interacting with soils and being lost from suspension or interactions between the SWW components and dissolved organic matter (DOM) in soil suspension; this characteristic has been identified for API-DOM interactions and metals in literature (Hernandez-Ruiz et al. 2012; Cohen et al. 2014).

As expected, due to the use of a buffer in the SWW, the pH was stable (Figure 5.4). The addition of the SWW to the soils generally increased the pH by a small amount

due to the buffering capacity of the SWW being greater than that of the soils, but variation over time was still observed (Borgman et al. 2013). Increased pH of soils after wastewater irrigation has been found to solubilise organic matter in the soils, increasing the concentration measured in filtrate. Increases in pH may increase the solubility of organic carbon due to an increased dissociation of the acid functional group (Andersson et al. 2000). The concentration of DOC in the liquid phase will have also increased from DOM in wastewater (Müller et al. 2007). The pattern of pH change over the shaking period varied between the soils. Sandy loam in SWW showed a similar pattern to the soil in 10 mM $CaCl_2$ solution where there was an initial small drop (0.4 pH units) before increasing to an observed maximum at 72 hours. The loam soil with SWW showed very little change overall with a pH increase of 0.1 over 120 hours. As discussed in the Chapter 4 soil pH is an important factor in the fate of APIs in soils, as it impacts the charge of the molecules and this in turn affects how it interacts in these soil environments. pH changes after irrigation of soils with wastewater is likely to vary down the soil profile with the most impact on pH being in the top layer of the soils (Borgman et al. 2013). It is also very variable over time and several researchers have reported variable pH response in soils after irrigation with increases, decreases and no change reported (Mohammad Rusan et al. 2007; Kalavrouziotis et al. 2008; Christou et al. 2017). This leads to difficulties when creating predictive models of APIs in wastewater-irrigated soils as effects appear to be dependent on soil properties, wastewater properties and frequency of application.

The DOC concentration in SWW showed an overall decrease of 65.7 % after 72 hours of shaking; this decrease has been attributed to biodegradation (Saadi et al. 2006; Tang et al. 2017). The experiment was not conducted under abiotic conditions, even though the sludge biotic input was denatured, so there are likely to be microorganisms present that could use the SWW carbon source as food, thereby reducing the concentration. DOM, usually quantified as DOC, has been extensively studied in soils for

its biodegradation and sorption fate and it has been found to be highly influenced by the DOM and soil characteristics (Kalbitz et al. 2000). Its fate in wastewater irrigated soils has, however, not been studied in detail. (Saadi et al. 2006). One study found that over 7 days the DOC concentration in effluent samples inoculated with soil and effluent microorganisms decreased by 43 %, compared with sterile effluent samples where no decrease was observed, indicating the change in DOC concentration was the result of biodegradation and not precipitation or aggregation (Saadi et al. 2006). This study had no solids present so did not measure sorption of DOC to soil particles. As well as biodegradation, DOC may have sorbed to the soils during the shaking experiment (Müller et al. 2007), which could have included sorption to minerals via ligand exchange or to soil organic matter via hydrophobic interactions (Westerhoff et al. 2000). The sandy loam with SWW showed the greatest loss of DOC. This could be due to the lower DOC concentration in 10 mM $CaCl_2$ solution (3-9 mg L^{-1}) resulting in more sites available for the SWW DOC to sorb to (Figure 5.5).

5.5.2 API loss

All three APIs studied were stable in SWW over 120 hours of shaking (Figure 5.1), even though there were significant changes in the suspension matrix. The losses of propranolol from both soil suspensions with SWW, and nevirapine in loam, were significantly higher after the addition of SWW. Naproxen sorption did not vary significantly in either soil and nevirapine in sandy loam.

Increased sorption after wastewater irrigation has been measured in column experiments using the APIs ibuprofen, estradiol and estrone, behaviour which was attributed to the accumulation of organic matter in the soils forming complexes with the APIs (Durán–Álvarez et al. 2014). DOM has been shown to reduce pesticide mobility in

soils by increasing DOM complexation in solution which can then in turn be co-absorbed onto soils (Müller et al. 2007). This may have caused the differences in loss from suspension between that measured in 10 mM $CaCl_2$ and that in SWW which were most apparent in sandy loam for propranolol (Table 5.6). This soil had the lowest organic matter content before SWW addition, and the lower DOC concentration after SWW addition shows that DOC sorbed to the soils; this could, therefore, have added additional surface sites to the soil for the APIs to interact with and have led to the increased Log K_{oc} in most cases (Figure 5.5 and Table 5.6).

The fate of naproxen has shown to not always be effected by the addition of wastewater to soils (Durán-Álvarez et al. 2012). This study showed that naproxen acted differently in two different soil profiles, with similar K_d values for the upper layer of soils with and without wastewater and a greater difference in soils from deeper in the soil profile. Differences in the sorption of naproxen were attributed to variations in soil organic matter quality. Long-term irrigation of soils with wastewater has been shown to have little impact on some negatively charged or neutral APIs (Dalkmann et al. 2014). This lack of change could have driven by these compounds not sorbing strongly to soil organic matter due to charge repulsion with negatively charged organic matter being dominant. Therefore, on addition of more organic matter from wastewater there was no significantly greater chance of sorption occurring to these sites at the experimental pH.

The data collected here suggests that, for some APIs, current methods will likely underestimate the partitioning of APIs to soil from wastewater irrigation as these experiments showed that SWW addition increased the loss of APIs from solution to soils in most cases compared with that in 10 mM $CaCl_2$. Whilst retarding the movement of APIs and other wastewater-derived contaminants reduces the risk to groundwater and streams, it may have an impact on the flora and fauna in soils that could be exposed to

greater concentrations of contaminants depending on bioavailability (Jager et al. 2005; Carter et al. 2014a).

The use of the OECD 106 methodology using a 'slurry' or suspension of soils in a liquid phase may not accurately represent the 'real world' situation when wastewater or any other contaminated waters are used for irrigation. There have been experiments undertaken that indicate variations in this ratio influences the sorption coefficient (Kah et al. 2007a; ElGouzi et al. 2012). The shaking of the sample tubes may also lead to greater adsorption compared to a static sample as the majority of the sorption sites are always in contact with the liquid phase (Kah et al. 2007a). A saturated paste method potentially provides a more representative measurement of sorption in soils as it more closely represents water content of soils under field conditions (ElGouzi et al. 2012). This method, however, are more time-consuming and have the potential to more errors due to variability in sample handling. This suggests that a discussion may be required to decide on a method that is both reproducible and relevant to the situation in real world.

5.6 Conclusions

This chapter has demonstrated that SWW alters soil supernatant properties during the 120 hour shaking test and that this, in turn, can affect the extent of API sorption to soils. As mentioned in Chapter 4, soil physico-chemical properties are key to understanding the fate of chemicals in soils. Irrigation with wastewater changes some of the soil properties important for chemical fate (pH, organic matter content and addition of microorganisms), so these should be considered when assessing the risks of chemicals to the soil environment. Loss of SWW DOC over the length of the loss from suspension experiment probably can increase the partitioning of positively charged APIs, this was apparent for propranolol in sandy loam. If the API partitions to DOC as well as the soil, the mobility of the API can be reduced if this complex is then co-absorbed to soil surfaces.

The ionisation state of the API at the altered pH after irrigation was more important for the positively charged propranolol than it was for the negatively charged naproxen and neutral nevirapine. This suggests that the difference in soil organic matter and pH will have greater impact on positively charged APIs and potentially zwitterions. In some cases the addition of SWW increased the loss from solution and therefore increased K_d and Log K_{oc} during the 120 hour shaking experiment. This has implications on the current terrestrial risk assessment where the trigger value for a more detailed soil risk assessment is Log $K_{oc} > 4$. If the experiment is only performed in 10 mM $CaCl_2$, as is currently required, it could lead to unknown risks of APIs in wastewater irrigated soils not being taken into account. Also there has been some research into the OECD 106 guideline's representability in estimating the 'real world' sorption of chemicals to soils. If this method could be refined to be more realistic it would aid in the production of more robust risk assessments.

6 Conclusions and future work

6.1 Conclusions and environmental risk assessment recommendations

The overall aim of this study was to investigate how wastewater irrigation, which commonly occurs in arid LLMICs, affects the fate of APIs in soils, and how resulting data might direct improvements to the current terrestrial environmental risk assessments. This aim was achieved through a series of laboratory experiments, which developed and tested methodology as part of a robust approach for determining APIs in complex matrices (Chapter 2). This was followed by experiments into the loss of APIs from a 10 mM $CaCl_2$ solution solution in a soil:water suspension (Chapter 4) before the latter was replaced by SWW (Chapter 5). The use of SWW ensured that the matrix was constant across the experiments as real wastewater components vary over time and geographically. SWW ingredients can be varied to understand how different treatment levels might affect the fate of APIs in soils, reflecting variations in irrigation water quality in LLMICs. If APIs from wastewater irrigation are to be included in ERA then SWW should be considered to achieve consistency in approach.

To thoroughly understand the abiotic partitioning fate of APIs in soils, the potential for biodegradation needs to be eliminated; it is therefore necessary to have sterile soils with comparable physico-chemical characteristics. Chapter 3 investigated the challenges that sterilising soils for use in OECD 106 type experiments presents. All three sterilisation methods investigated led to changes in the soil matrix that meant a comparable sterile repeat of the loss from suspension experiment could not be undertaken in order to quantify biodegradation. Certain methods of sterilisation are often applied with the assumption that the method delivers unchanged, 'sterilised' soils with no experimental data reported to confirm this (Al-Rajab et al. 2010; Zhang et al. 2013). The data reported in Chapter 3 challenges this assumption, and indicates that, if a soil is to be accurately characterised,

then it should be tested for bacterial activity and for any changes to soil properties after sterilisation has been undertaken. Guidance should be provided to researchers and analysts to ensure that any changes to the soil matrix that do occur are quantified and deemed to be acceptable; these criteria will need to be defined. The recommendation from this research would be that gamma irradiation is the best option for soil sterilisation. When risk assessments are required to distinguish between biodegradation and sorption, the soils' sterility and physical characteristics should be checked. Parameters of interest include pH, particle size fractions, clay content, organic matter characteristics and leachable OC. These data should be reported along with the results from sorption experiments undertaken in the soils. There is the potential that sorption data could be corrected to include the solubilisation of OC and pH-related changes in ionisation of the APIs in K_d or Log K_{oc} calculations.

For some APIs and soils (naproxen in loam and sandy loam and nevirapine in sandy loam) no loss from solution was measured. However, for propranolol in both soils and nevirapine in loam, addition of SWW significantly increased the loss from solution, resulting in an observed increase of Log K_{oc} (increase of 0.4 in loam for both APIs and 0.7 for propranolol in sandy loam, Table 5.4). The increase in Log K_{oc} in SWW indicated that any fate assessment applied to areas where wastewater irrigation is used regularly might underestimate environmental risk if Log K_{oc} is not taken into account (Chapter 5). Increasing the sorption of APIs to soil particles will reduce the pore water concentration, potentially leading to greater persistence in the environment. This depends on changes to soil microbial populations from wastewater, which may affect biodegradation of APIs. Whilst retarding the movement of APIs and other wastewater-derived contaminants reduces the risk of transport to groundwater and streams it may increase impact on the flora and fauna in soils. Organisms may be exposed to greater bioavailable concentrations

of contaminants which are more persistent, though APIs strongly sorbed to soils may reduce risks to soil flora and fauna (Jager et al. 2005; Carter et al. 2014a).

There have been some queries into the use of Log K_{oc} for the risk assessment of ionisable compounds in soils as it was initially used to explain the partitioning behaviour of pesticides (ECETOC 2004). The main assumption is that the organic carbon content of the soil will be the dominant factor of adsorption of the chemical. Log K_{oc} was developed as a driver for understand the fate of neutral industrial chemicals in soils, though generic exposure pathways and modes of action and fate studies have focussed on neutral species (Tarazona et al. 2010). Any sorption or desorption coefficients calculated for ionisable compounds should be corrected to only include the unionised fraction (ECB 2002; Tarazona et al. 2010). This caveat is rarely acknowledged and is not part of the OECD 106 guideline; in fact, applying this correction will mean that all of the ionic interactions with soil, which have been shown to be important for APIs, will not be taken in to account. However, the data presented in Chapter 4 shows that once the K_d of the four APIs was normalised for the organic carbon content of the soils at equilibrium, there was variation between the soils, so other factors must be important during loss of APIs from solution. Organic matter characteristics are not consistent between soils (Chapter 1). This could be the source of some of the differences in the Log K_{oc} calculated as this assumes that all organic carbon is the same. For positively-charged APIs at environmental pH, such as propranolol, correcting for organic carbon does not cover all potential negatively charged sorption sites where ionic interactions can take place. Clay generally has a negative charge and a CEC that would promote sorption of positively-charged APIs (Lees et al. 2016). Negatively-charged APIs, such as naproxen, can establish π-π bonds with humified organic matter depending on the structure of the molecule. These bonds are weak due to the repulsion from negatively-charged soil particles, however, they may also bond to positively charged sites on clays by non-specific electrostatic interaction

(ECETOC 2004; Durán-Álvarez et al. 2015). Sorption coefficients were developed for neutral molecules; however, in this circumstance the Log K_{oc} correction did not explain all sorption for nevirapine (neutral at experimental pH) (Table 4.9). Log K_{oc} also implies that the parameter is consistent across the matrices being assessed (e.g. sludge, sediment, soil). This is untrue for polar chemicals as ionic interactions will become more important, and the accuracy of the Log K_{oc} estimations declines as a result (ECETOC 2004). As Log K_{oc} does not include electrostatic interactions clays and other soil surfaces, it should perhaps be replaced by K_d as a conceptual model as this parameter includes all interactions. Reassessment of the trigger value for an extended risk assessment of ionisable chemicals in soils would appear necessary in order to fully take into account all processes controlling the fate in soils as currently this trigger is only valid in sewage sludge. This could include clay, as a potential sorption site, variations in organic matter properties in soil and the ionisation state of the API (charge and fraction ionised). For areas were wastewater irrigation is used developing a extended TERA, i.e. a risk assessment that includes Phase IIb without the need for a surface water trigger value, is essential.

The matrix characteristics were found to vary throughout both loss from suspension experiments and the desorption experiment; namely pH and DOC concentration. Both of these parameters impacted on the fate of the APIs. pH variation changed the fraction of API ionised at times throughout the experiments and changed the pH dependant charge on organic matter, clay minerals and metal sesquioxide components of soil (Hyun et al. 2004; Lees et al. 2016). Changes to the ionised fraction of the four APIs had the potential to be most important for ofloxacin, in the range of pH measured throughout these experiments, due to its zwitterion properties (Figure 4.1). It however was not impacted by the change of pH as sorption was both extensive and rapid (> 99 % adsorbed in under 6 hours). APIs have been found to associate with DOC, increasing their mobility

compared to those sorbed to soil particles (Tolls 2001). DOC may also sorb to soils during the shaking experiments, which will reduce the mobility of APIs associate with the DOC (Müller et al. 2007). The change in the matrix DOC and pH during sorption experiments appears to be both unreported and novel, and the data from this study suggest that monitoring of both parameters should be included in future OECD 106 type tests. Depending on the time an API takes to reach equilibrium during the OECD 106 test, changes in pH and DOC concentration could influence fate and will vary between soil types, making any assumptions extrapolated from the data uncertain (such as Log K_{oc}). This could then lead to errors in reporting the risks associated with a chemical in soils. In 'real world' situations where land is irrigated with wastewater, some of the tests proposed in the existing ERA process may not reflect actual conditions. Due to the constant input of irrigation water throughout the growing season, depending on weather and crop type, equilibrium partitioning of an API between soils and pore water may never be reached. There will also be constant changes to soil matrices through variations in DOC, pH and other nutrients and contaminant addition. Risks to environmental compartments may not be accurately reported if all tests rely on an equilibrium partition coefficient; sorption-desorption tests may need to be altered to include this scenario.

The limitations to the current European Medical Agency ERA for APIs identified in this thesis and recommendations for their application by LLMICs are shown in Table 6.1. While there appear to be many improvements identified, most relate to the fact that wastewater irrigation, as a source of APIs to soils, is not included in the current ERA. Additionally, there are recommendations for transferring the European ERA to LLMICs situations, which include scenarios not identified in Europe, such as using wastewater for irrigation.

Table 6.1 - Limitations to current European ERA for APIs identified in this thesis (highlighted) and recommendations for different global regions (EMEA 2006)

Phase	Data required to define exposure	Limitations to current ERA	Suggestions for inclusion of wastewater in TERAs
Phase I – estimation of exposure	Predicted environmental concentration (PEC) for aquatic compartment PBT* assessment if Log K_{ow} >4.5 Tailored ERA if mode of action is of concern	- PEC restricted to aquatic compartment - PEC calculated using maximum daily dose consumed per inhabitant which is difficult for LLMICS (Chapter 1) and amount of wastewater per inhabitant estimated from TGD* which may not tie across to LLMIC situation	- Include a soil ecotoxicology trigger for soil compartments with wastewater irrigation as this will identify the need for an extended TERA* - Daily dose data cannot be gathered from prescriptions in LLMICs, as there are many unauthorised pharmacies and inconsistent adherence to therapeutic treatments (Kookana et al. 2014) (Chapter 1). Also wastewater in LLMICs is often not official so calculating wastewater per inhabitant is difficult. Other ways of calculating exposure will be required in these areas, e.g. by usage tonnage and Log K_{ow} - Develop a model for wastewater irrigation scenarios to estimate sorption and soil persistence

Phase II A – Initial environmental fate and effects analysis	Ready biodegradability Water/ sediment aerobic/anaerobic transformation Adsorption - desorption using a batch equilibrium method Toxicity to algae, *Daphnia sp.* and fish Log K_{oc} assessment (> 4 TERA* required) Activated sludge respiration inhibition	- The EMA guidelines only include sludge for all of the tests but the OECD 106 method is designed for soils and is a recommended test - OECD 106 guideline misses some instructions that have been mentioned throughout this thesis and would aid the use in LLMICs (filter selection, sterile soil methods, DOC and pH changes) - Wastewater irrigation impact on fate of APIs is not included - Log K_{oc} is focussed on sorption to sewage sludge - Log K_{oc} calculated from sorption experiments in 10 mM $CaCl_2$ solution may underestimate the risks from wastewater irrigation	- Focus needs to be on soils as well as sludge due to the addition of APIs from wastewater irrigation, Log K_{oc} trigger is not relevant for wastewater irrigated soils (Chapters 5 and 6) - Recommend a method to sterilise soils, (Chapter 3), and add to OECD 106 guideline to ensure that the soils have actually been successfully sterilised with minimal changes to soil matrix - Additional information that would provide researchers more opportunities to compare data would include measuring soil matrix properties throughout the length of the sorption experiment (Chapter 4) - Develop and implement a method that uses a more realistic porewater- soil ratio rather than the OECD 106 (Chapter 5)

Phase II B – Extended environmental fate and effects analysis (TERA)*	Nitrogen transformation test in soil Aerobic and anaerobic transformation in soil Plant growth Earthworm acute toxicity *Collembola* reproduction test	- Whilst none of these were studied during this thesis in particular all of these tests could be modified to include wastewater irrigation scenarios

*PBT – persistent, bioaccumulating or toxic, TGD – technical guidance document (ECB 2002), TERA – terrestrial environmental risk assessment

6.2 Future research recommendations

As a result of this study, several gaps in the current knowledge of API behaviour in soils from wastewater irrigation have become obvious. Along with the improvements suggested for the ERA of APIs in LLMICs using wastewater for irrigation (Table 6.1), additional research could be undertaken to understand the fate of APIs in these environments.

The objectives of this PhD were not fully met due to instrument and time limitations in the laboratory (Section 1.6). These included; varying the wastewater quality used in the modified OECD 106 study, column dissipation studies with different irrigation regimes and using soils sourced from LLMICs and compare them to European studies. Completing these aims would provide a good basis of data to use to understand what influences the sorption fate of APIs in soils in areas with high wastewater irrigation.

Varying the wastewater matrix in the soil sorption and desorption experiments would aid understanding of how different qualities of processed and unprocessed wastewater may affect the sorption fate in soils. For example, using non-freeze dried and baked sewage at the start would increase the number of microorganisms present, this could could increase biodegradation of APIs during the experiment. The concentration of metal ions could be varied as these have been shown to have an impact on the stability and sorption fate of fluoroquinolone antibiotics in wastewater and other environmental matrices (Aristilde et al. 2008; Aristilde et al. 2016). Finally, the pH of the SWW could be varied, wastewater generally has a constant pH around 7.5 but this type of experiment could provide information on worst-case or extreme scenarios (Table 5.2).

To understand the influence of wastewater irrigation on API fate during a crop cycle, soil dissipation columns irrigated with SWW contaminated with APIs should be studied using a range of soil types common in LLMICs, along with APIs in common use

by these countries' populations. This will provide information on the mobility of APIs through the soil profile and an understanding of the possible contamination of groundwater from infiltration, it is a potential extension to the OECD 106 method that improves realism. Some column studies have already been undertaken, some with real wastewater, and at different soil depths, but these have differing outcomes suggesting that more research is needed (Chefetz et al. 2008; Borgman et al. 2013; Durán–Álvarez et al. 2014; Vogel et al. 2014). The studies found that the changes to soil matrices identified in this thesis, such as pH and organic matter addition, had impacts on the fate of APIs. The dominant factor affecting fate appeared to vary between studies with some indicating that pH was important (Borgman et al. 2013), or soil organic matter (Chefetz et al. 2008) and organic matter from wastewater irrigation water (Durán–Álvarez et al. 2014). Others have used field sites, comparing API concentrations in wastewater-irrigated soils and groundwater-irrigated soils (Chen et al. 2011). Although Chen (2011) found that the concentration of APIs was generally higher in wastewater-irrigated soils than groundwater-irrigated soils, no mechanisms were discussed. SWW-irrigated soil columns will provide more data and understanding on how fate of APIs is influenced by wastewater irrigation during crop growing seasons. This could then incorporated within environmental risk assessments to ensure that Log K_{oc} reflects the 'real world' situation (Table 6.1).

Using soils sourced from LLMICs for the sorption-desorption experiments with and without wastewater will provide information on how different soils with potentially different organic carbon may affect API fate. The organic matter composition in these soils will differ from those in higher income countries due to the way organic matter is produced and degraded in soils. It is composed of many different organic materials ranging from biopolymers (e.g. polysaccharides, lipids, proteins and lignin), humic substances derived from biopolymers and diagenetically matured kerogen and

combustion-related black carbon or char materials (Aiken et al. 1985; Huang et al. 2003). The different combination of these organic materials will impact the rate and strength of sorption of any organic compound to the soil organic matter (Huang et al. 2003). This data could then be used to produce models and tailor-made risk assessments for the sorption fate of APIs in LLMICs.

Finally, by gathering fate data on a range of APIs and soils will provide a global database with shared costs. A database could be used to understand the mechanisms of sorption, biodegradation, and other fate characteristics that mechanistic studies using fewer APIs struggle to provide. While small studies are beneficial because a thorough, detailed assessment can be undertaken, it makes it difficult to tease apart the drivers of API fate. If these data were gathered into an open access resource, it would allow more detailed modelling to take place. Databases such as this could be then used to estimate fate data of similar non-API compounds and read across to other APIs within the same physico-chemical class. Industry collaboration would aid the formation of such a database due to the requirements for all new medicines post-2006 requiring an environmental risk assessment for marketing authorisation. A collaborative approach is already being undertaken with the iPiE project (intelligence-led assessment of pharmaceuticals in the environment) which includes several pharmaceutical companies, universities and research institutes (iPiE 2017).

6.3 Final words

Ultimately, the fact that the input of chemicals from wastewater irrigation is not included in current environmental risk assessments is an oversight that requires consideration, given the increased use of pharmaceuticals in LLMICs and health and ecosystems concerns. Irrigation with wastewater provides continuous inputs of chemicals into soils throughout the growing season so it is vital that more work is done to understand

the ultimate fate of pollutants in soil introduced through this practice. In LLMICs where wastewater treatment is not used or is of a basic quality, there may be a greater input of APIs and other chemicals to soils which, as of yet, have not been included in risk assessments. The use of Log K_{oc} for triggering the extended terrestrial risk assessment does not include all of the potential mechanisms of loss of APIs from soil suspensions and assumes all sources of OC are consistent in all matrices being assessed. This oversight risks not fully taking into account the fate of APIs in soils in ERA.

Wastewater has the potential to change the fate of chemicals in soils meaning that current risk assessments may underestimate risks involved. It is worth remembering that in many LLMICs risk assessments may not be the priority as clean water and a stable food supply are of greater concern. Wastewater irrigation is used globally, not just in LLMICs, so developing thorough ERAs to include it in higher income countries first; the ERA framework can be transferred to LLMICs when the time is right.

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